ional Institute on Alcohol Abuse and Alcoholism

RESEARCH Monograph-6

Development of Animal Models as Pharmacogenetic Tools

DEPARTMENT OF HEALTH AND HUMAN SERVICES • Public Health Service • Alcohol, Drug Abuse, and Mental Health Adminis

National Institute on Alcohol Abuse and Alcoholism

RESEARCH MONOGRAPH SERIES

1. **Alcoholism and Alcohol Abuse Among Women: Research Issues**

   Proceedings of a Workshop on Alcoholism and Alcohol Abuse Among Women, Jekyll Island, Georgia, April 2–5, 1978. This workshop was the first NIAAA-sponsored gathering of researchers to discuss the state of the art concerning women and alcohol. Workshop objectives were to discuss existing research, identify key issues, and develop research priorities for NIAAA research efforts.

   DHHS Pub. No. (ADM) 79–835 — *Printed 1980*
   GPO Stock No. 017–024–00988–1 — *Cost: $5.50*

2. **Alcohol and Nutrition**

   Editors: Ting-Kai Li, M.D., Steven Schenker, M.D., and Lawrence Lumeng, M.D.

   Proceedings of a Workshop on Alcohol and Nutrition, Indianapolis, Indiana, September 26–27, 1977. The proceedings address alcohol metabolism and important questions related to the effects of nutrition on enzyme and cofactor levels in alcohol oxidation.

   DHHS Pub. No. (ADM) 79–780 — *Printed 1979*
   GPO Stock No. 017–024–00954–7 — *Cost: $6.00*

3. **Normative Approaches to the Prevention of Alcohol Abuse and Alcoholism**

   Editors: Thomas C. Harford, Ph.D., Douglas A. Parker, Ph.D., and Lillian Light.

   Proceedings of a symposium, San Diego, California, April 26–28, 1977. Papers describe the theoretical and empirical frameworks of the sociocultural and distribution-of-consumption models of drinking behavior and assess their implications for strategies of primary prevention.

   DHHS Pub. No. (ADM) 79–847 — *Printed 1980*
   GPO Stock No. 017–024–00984–9 — *Cost: $5.00*

4. **Services for Children of Alcoholics**

   This monograph is the product of a symposium held in Silver Spring, Maryland, September 24–26, 1979. A representative sample of professionals in the alcoholism field was invited to share their experiences and insights in this area and to identify critical policy issues, and to assess future needs.

   DHHS Pub. No. (ADM) 81–1007 — *Printed 1981*
   GPO Stock No. 017–024–01058–8 — *Cost: $5.00*

(Continued on inside back cover)

Research Monograph No. 6

DEVELOPMENT OF ANIMAL MODELS AS PHARMACOGENETIC TOOLS

Proceedings of a Workshop
December 4-6, 1978
Boulder, Colorado

Sponsored by:

The Alcohol Research Center of the University of Colorado and the National Research Centers Branch, NIAAA

Edited by:

Gerald E. McClearn
Richard A. Deitrich
V. Gene Erwin
of the University of Colorado Alcohol Research Center

NATIONAL INSTITUTES OF HEALTH
NIH LIBRARY
SEP 2 6 2007
BLDG 10, 10 CENTER DR
BETHESDA, MD 20892-1150

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration

National Institute on Alcohol Abuse and Alcoholism
5600 Fishers Lane
Rockville, Maryland 20857

This publication is the product of a research workshop on the development of animal models as pharmacogentic tools held in Boulder, Colo., Dec. 4-6, 1978, under grant P50 AA 03527 awarded by the National Institute on Alcohol Abuse and Alcoholism to the University of Colorado Alcohol Research Center. The presentations herein are those of the listed authors and may not necessarily reflect the opinions, official policy, or position of the National Institute on Alcohol Abuse and Alcoholism; the Alcohol, Drug Abuse, and Mental Health Administration; the Public Health Service; or the U.S. Department of Health and Human Services.

The National Institute on Alcohol Abuse and Alcoholism has obtained permission from the copyright holders to reproduce the figures and tables on pp. 1B1, 1B2, 1B3, 1B4, 1B5, 1B6, 1B7, 1B8, 1B9, 1B10, 1B11, 1B12, 2D1, 2F1, 2F3, 2F4, 2F5, 2F6, 2F7, and 2G1. Further reproduction of these materials is prohibited without specific permission of the copyright holder. Adapted tables of copyrighted materials may be copyrighted; thus before reprinting, readers are advised to determine the copyright status or to secure permission of the copyright holder. All other material contained in the monograph, except quoted passages from copyrighted sources, is in the public domain and may be used and reprinted without permission. Citation of the source is appreciated.

The U.S. Government does not endorse or favor any specific commercial product or commodity. Trade or proprietary names appearing in this publication are used only because they are considered essential in the context of the studies reported herein.

Library of Congress Catalog Card Number: 81-600126

DHHS Publication No. (ADM) 81-1133
Printed 1981

For sale by the Superintendent of Documents, U.S. Government Printing Office,
Washington, D.C. 20402

Foreword

This volume is another in a series of research monographs by the National Institute on Alcohol Abuse and Alcoholism. The series presents current information on a number of topics relevant to alcohol abuse and alcoholism as reported through workshops in research, prevention, and treatment, and through state-of-the-art reviews of selected subjects.

The Institute has a longstanding commitment to disseminate information broadly from researcher to researcher in order to promote new advances in knowledge about alcoholism. These proceedings are another link in the continuing process of keeping active researchers well informed about the methods and tools useful in conducting quality research.

It is our hope that scientists, and others interested in alcohol research, will find this monograph informative about the effects of alcohol, and useful in shedding light on some of the complexities faced by alcoholism researchers.

John R. DeLuca
Director
National Institute on Alcohol Abuse
and Alcoholism

Preface

This monograph reports the proceedings of a research workshop held in Boulder, Colorado, December 4-6, 1978, co-sponsored by the University of Colorado Alcohol Research Center and the National Institute on Alcohol Abuse and Alcoholism (NIAAA). The Alcohol Research Center is one of nine National Alcohol Research Centers funded by the NIAAA.

The research of each Center focuses on a central theme of importance to the study of alcoholism, ranging from the neurophysiological effects of alcohol consumption to the epidemiological factors associated with alcoholism. In addition to their primary mission of developing new knowledge through original research in their chosen areas within the field of alcoholism, the Centers are responsible for disseminating that knowledge broadly to the scientific and lay communities. In this connection, the Centers have joined the NIAAA in organizing and holding workshops and conferences on topics close to their specialty The proceedings in this volume represent one of these collaborative activities.

The purpose of the Boulder workshop was to survey the animal literature in pharmacogenetics generally, and in behavioral pharmacogenetics specifically, including the work on genetic influences on individual differences in the self administration of various substances. In addition, it was intended that behavioral and pharamcological phenotypes be identified such that their explication would have maximum impact on understanding the use and abuse of alcohol and other substances. Finally, it was hoped that one outcome of the workshop would be the design of a systematic and coordinated program for the screening of the available stocks and strains of animals and the formulation of a program of selected breeding for high priority phenotypes.

As a direct result of this research workshop, the NIAAA, with the collaboration of the Division of Research Services of the National Institutes of Health has developed a heterogeneous stock of rats that will be invaluable in facilitating alcohol research and in contributing to our knowledge about the range of responses to the consumption of alcohol. Thus, this monograph, it is hoped, will call attention to the power of selective breeding and the use of properly maintained stocks of experimental animals as a important tool in developing our scientific knowledge.

Albert A. Pawlowski, Ph.D.
Chief,
National Research Centers Branch
Division of Extramural Research
National Institute on Alcohol Abuse
and Alcoholism

Introduction

Pharmacogenetics may be a small stream in the torrent of biomedical research, but its importance is disproportionately large. First, pharmacogenetics provides a perspective on individuality of drug response. This individuality has profound implications both for the conduct and interpretation of basic and applied research and for the clinical practice of medicine and pharmacy, where treatment is, after all, administered to individuals. Second, pharmacogenetics offers the prospect, through manipulation of individuality in drug-related processes, of generating animal models of great precision and power. It is this prospect that has led to the conference whose proceedings are reported in this monograph.

From the early discoveries of single-gene influence on response to succinylcholine and primaquine, pharmacogenetics has expanded its empirical base and its theoretical conceptions so that we now have the outline of a picture of ubiquitous genetic influence on all aspects of drug action—uptake, distribution, disposition, and target organ sensitivity—for the gamut of substances—from the traditional nostrums of folklore through patent medicines and prescription drugs to substances of abuse. The muscle of detail is being added very rapidly to this skeletal outline.

Much of the empirical base for this view of pervasive genetic influence has come from animal studies. Although there is continuing debate over the pertinence of particular models, it seems apparent that laboratory animals, in pharmacogenetics as in pharmacology itself, will continue to provide a large share of knowledge. It is probably fair to state, however, that the implications of pharmacogenetics are only gradually filtering into and having an effect upon pharmaceutical sciences.

Nevertheless, there is a strong presumption by those already in the area that the use of genetic control will grow in a positive feedback fashion. As the perception spreads that studies using genetic control have increased efficiency and power, more and more researchers will wish to avail themselves of these useful tools, thus increasing the evidential base for the effectiveness of genetic control and attracting converts. It is not at all unlikely that, within a few years, research using unspecified or haphazardly maintained animals will be completely unacceptable for publication.

The increased demand for genetic tools imagined in this scenario might find the supply wanting. The time is ripe to prepare systematically the genetic tools that will soon be demanded by the pharmaceutical sciences.

This view was a major theme that emerged from a workshop held in 1976 at the University of Colorado (Boulder) under the auspices of the Social Science Research Council, and it was presented as a topic for discussion to the Committee on Substance Abuse and Habitual Behavior of the National Research Council. That body approved a plan to convene a conference on the matter, and funds were obtained from the National Institute on Alcohol Abuse and Alcoholism through the Alcohol Research Center at the University of Colorado.

Gerald E. McClearn
Director
Institute for Behavioral Genetics
University of Colorado (Boulder)

Conference Participants*

Michael B. Bass, Ph.D.
Center of Alcohol Studies and Department of Psychology
Rutgers University
New Brunswick, N.J. 08903

Jules Cass, Ph.D.
Veterans Administration Hospital
Washington, D.C. 20422

Allan C. Collins, Ph.D.
School of Pharmacy and Institute for Behavioral Genetics
University of Colorado
Boulder, Colo. 80309

J.C. DeFries, Ph.D.
Institute for Behavioral Genetics and Department of Psychology
University of Colorado
Boulder, Colo. 80309

Richard A. Deitrich, Ph.D.
Department of Pharmacology
University of Colorado Health Sciences Center
Denver, Colo. 80262

Carlton K. Erickson, Ph.D.
College of Pharmacy
University of Texas
Austin, Tex. 78712

C.J. Peter Eriksson, Ph.D.
Department of Pharmacology
University of Colorado Health Science Center
Denver, Colo. 80262

Kalervo Eriksson, Ph.D.
Research Laboratories of the State Alcohol Monopoly (Alko)
Helsinki 10, Finland SF-0011

V. Gene Erwin, Ph.D.
School of Pharmacy and Institute for Behavioral Genetics
University of Colorado
Boulder, Colo. 80309

Eugene Fleming, Ph.D.
Department of Psychiatry and Human Behavior
University of California
Irvine, Calif. 92717

Benson E. Ginsburg, Ph.D.
Department of Biobehavioral Sciences
University of Connecticut
Storrs, Conn. 96268

Dora B. Goldstein, M.D.
Department of Pharmacology
Stanford University School of Medicine
Palo Alto, Calif. 94304

Gary P. Horowitz, Ph.D.
Department of Psychology
State University of New York
Binghamton, N.Y. 13901

Janet Shibley Hyde, Ph.D.
Psychology Department
Bowling Green State University
Bowling Green, Ohio 43403

Ryoko Kakihana, Ph.D.
Department of Biological Sciences
San Francisco State University
San Francisco, Calif. 94132

David Lester, Ph.D.
Center of Alcohol Studies and Department of Psychology
Rutgers University
New Brunswick, N.J. 08903

---

* Affiliations reflect status as of the time of the workshop, December 1978.

Peter K. Levison, Ph.D.
Committee on Substance Abuse and Habitual Behavior
National Research Council
Washington, D.C. 20418

Ting-Kai Li, M.D.
Departments of Medicine and Biochemistry
Indiana University School of Medicine and Veterans Administration Hospital
Indianapolis, Ind. 46223

Carol B. Lynch, Ph.D.
Department of Biology
Wesleyan University
Middletown, Conn. 96457

Richard Marcus, Ph.D.
Extramural Research Branch
National Institute on Alcohol Abuse and Alcoholism
Rockville, Md. 20857

Stephen C. Maxson, Ph.D.
Department of Biobehavioral Sciences
University of Connecticut
Storrs, Conn. 96268

Gerald E. McClearn, Ph.D.
Institute for Behavioral Genetics
Departments of Psychology and Biology and School of Pharmacy
University of Colorado
Boulder, Colo. 80309

William Meredith, Ph.D.
Department of Psychology
University of California
Berkeley, Calif. 94720

Albert A. Pawlowski, Ph.D.
Extramural Research Branch
National Institute on Alcohol Abuse and Alcoholism
Rockville, Md. 20857

Dennis R. Petersen, Ph.D.
School of Pharmacy and Institute for Behavioral Genetics
University of Colorado
Boulder, Colo. 80309

Patricia Renaldi, Ph.D.
Department of Psychiatry and Human Behavior
University of California
Irvine, Calif. 92717

R.C. Roberts, Ph.D.
ARC Unit of Animal Genetics
Institute of Animal Genetics
Edinburgh, Scotland EH9 3JN

David H. Ross, Ph.D.
Department of Pharmacology
University of Texas Health Science Center
San Antonio, Tex. 78284

G.C. Salmoiraghi, M.D.
National Institute on Alcohol Abuse and Alcoholism
Rockville, Md. 20857

Boris Tabakoff, Ph.D.
Department of Physiology and Biophysics
University of Illinois Medical Center
Chicago, Ill. 60680

Ronald G. Thurman, Ph.D.
Department of Pharmacology
School of Medicine
University of North Carolina
Chapel Hill, N.C. 27514

James R. Wilson, Ph.D.
Institute for Behavioral Genetics and Departments of Psychology and Biology
Boulder, Colo. 80309

Table of Contents

Page
Foreword iii
Preface iv
Introduction
Gerald E. McClearn vi
Conference Participants viii

Section I. Current Perspectives on Selective Breeding
Introduction
Gerald E. McClearn 3
Current Perspectives on Selective Breeding: Example and Theory
J.C. DeFries 11
Current Perspectives on Selective Breeding: Theoretical Aspects
R.C. Roberts 37
A Review of Selective Breeding Programs
Janet Shibley Hyde 59

Section II. Selective Breeding for Alcohol-Related Phenotypes
Introduction
Gerald E. McClearn 81
Finnish Selection Studies on Alcohol-Related Behaviors: General Outline
Kalervo Eriksson and Maija Rusi 87
Finnish Selection Studies on Alcohol-Related Behaviors: Factors Regulating Voluntary Alcohol Consumption
C.J. Peter Eriksson 119
Selective Breeding for Ethanol Sensitivity: Short-Sleep and Long-Sleep Mice
Gerald E. McClearn and Ryoko Kakihana 147
A Review of Research Using Short-Sleep and Long-Sleep Mice
Allan C. Collins 161

Indiana Selection Studies on Alcohol-Related Behaviors
T.-K. Li, L. Lumeng, W.J. McBride, and M.B. Waller... 171
Selective Breeding for Ethanol Sensitivity: Least Affected and Most Affected Rats
M.B. Bass and D. Lester.. 193

Section III. Pharmacogenetic Phenotypes

Introduction
Gerald E. McClearn.. 205
Pharmacogenetic Models and Behavioral Responses to Opiates
Gary P. Horowitz... 209
What Traits To Breed For and How To Measure Them
Dora B. Goldstein 233
Multivariate Methodology
William Meredith... 249

Section IV. Reports of the Task Groups

Introduction
Gerald E. McClearn.. 269
The Design of Selection Experiments................................ 271
Phenotypes To Be Selected for Breeding Purposes in Alcohol Research.. 279
Coordination of Participating Pharmacological Laboratories 285
Coordination of Participating Genetics Laboratories............ 287
Concluding Comment.. 291
Glossary 293
Index.. 297

SECTION I: CURRENT PERSPECTIVES ON SELECTIVE BREEDING

Introduction

Gerald E. McClearn

This monograph focuses on the use of selective breeding to generate animal models for research on alcohol and other drugs. It is possible, of course, to carry out a successful selective breeding program with no understanding of the underlying genetic system. Domestication of dogs, cattle, horses, corn, wheat, and most other agriculturally essential animals and plants was accomplished by strong arm selective breeding long before Mendelian rules were known, let alone the quantitative model that generalized the Mendelian single-gene theory to complexly determined systems.

Quite obviously, we should approach the issue of pharmacogenetic models armed with the best of current theory and informed of the empirical matrix to which the theory relates. To do otherwise would be grossly inefficient and would invite serious mistakes both in generating the animal models and in interpreting the results of research conducted with them. The names of Chetverikov, Fisher, Haldane, and Wright loom large in the history of the development of quantitative genetic theory—a theory that reconciled the fields of evolutionary biology and genetics as well as the Mendelian and the biometric views of inheritance. Among other current textbooks on the subject, the work of Falconer (1960) is the standard classic representation of this quantitative model. Excellent shorter presentations have been made by two of the authors of chapters in this first section, J.C. DeFries (in McClearn and DeFries 1973, ch. 9) and R.C. Roberts (1967). The reader who is being exposed to these notions for the first time could find no more lucid introductions than those provided by DeFries and Roberts in this monograph, where they concentrate on those aspects of the theory that are particularly relevant to selective breeding.

For readers who prefer to be led gently into algebraic matters, the following graphic representation of some of the basic notions is presented. Terms introduced in this section are defined in the glossary.

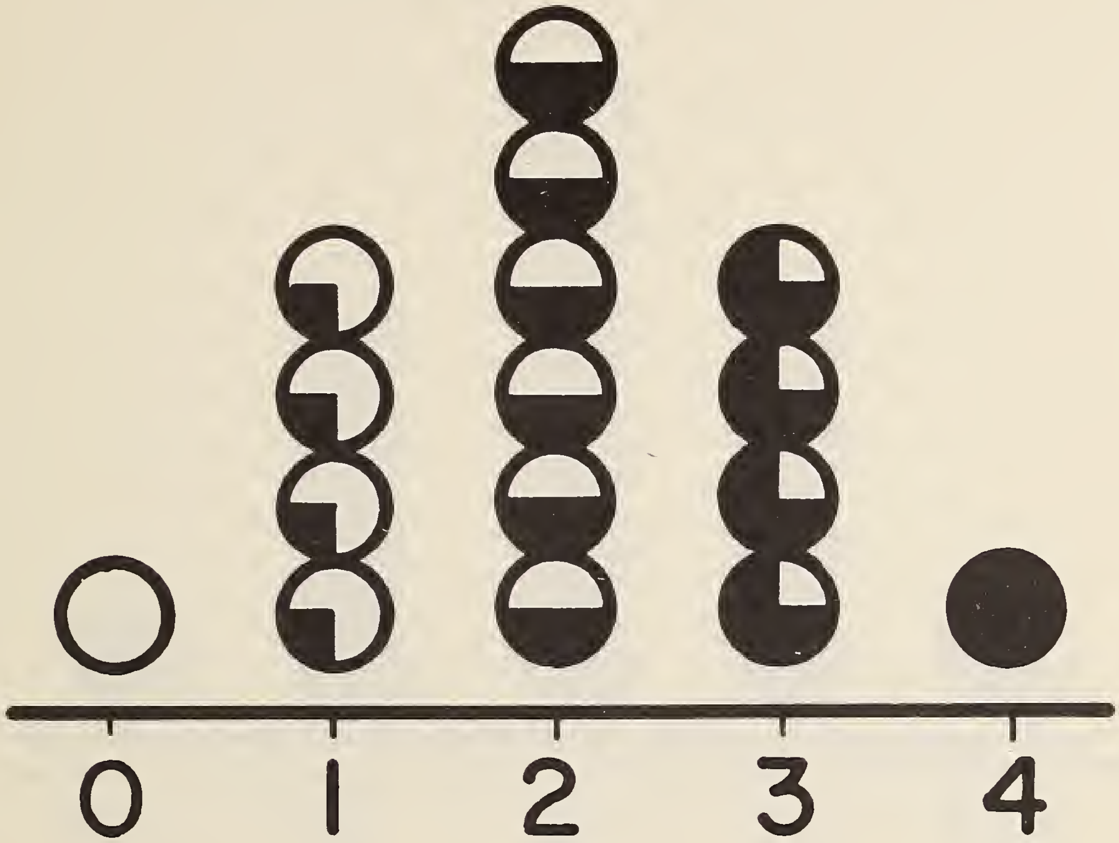
Let us imagine that there exists in a population a number of gene loci that influence the phenotype (trait) under investigation. Furthermore, let us represent the number of "+" alleles at these loci (the alleles' making for higher phenotypic scores) by differentially filling in the circle that represents an individual organism. Thus, an individual designated by an open circle has no + alleles, only - ones. An individual designated by a filled circle has all possible + alleles. An individual indicated by a half-filled circle has one-half of all possible + alleles, and so on. Now we can locate each individual on a phenotypic dimension as shown in figure 1. It can be seen that there is an exact correspondence between the percentage of + alleles and the phenotypic score. In figure 2, the correspondence is weaker. On the average, individuals with a higher percentage of + alleles are above the phenotypic mean and vice versa, but it is clear that the genetic factors alone are insufficient to describe the variability of the population. The other domain of factors that influences phenotypic score is, of course, that of environmental influences. In figure 3, the relationship of genotype to phenotype is even weaker.

The basic concept applicable to this situation is that of *heritability.* As we shall see, this term has both broad and narrow definitions. At present, it suffices to know that heritability, in its broad sense, is the percentage of total phenotypic variance in a population that is attributable to genetic variation. In figure 1, heritability is 1.00, and it has smaller values in figures 2 and 3, respectively. In the case of an absence of heritability (i.e., a phenotype for which all variance in the population being examined is due to environmental factors), there are no + or - alleles; by definition, loci affecting the trait do not exist.

It is important to note that heritability is a descriptive term. In another population with different allelic frequencies, or in a different range of environmental circumstances, the heritability of the trait might be different.

With respect to selective breeding, the basic operation is that of mating males and females of similar phenotypic scores. If we wish to generate a line of animals with high scores on the phenotype shown in figure 1, we might breed only those above the cutoff line shown in figure 4. It is clear that these individuals, who will be the exclusive parents of the next generation in that line, carry forward a heavy representation of + alleles for their progeny. These progeny should

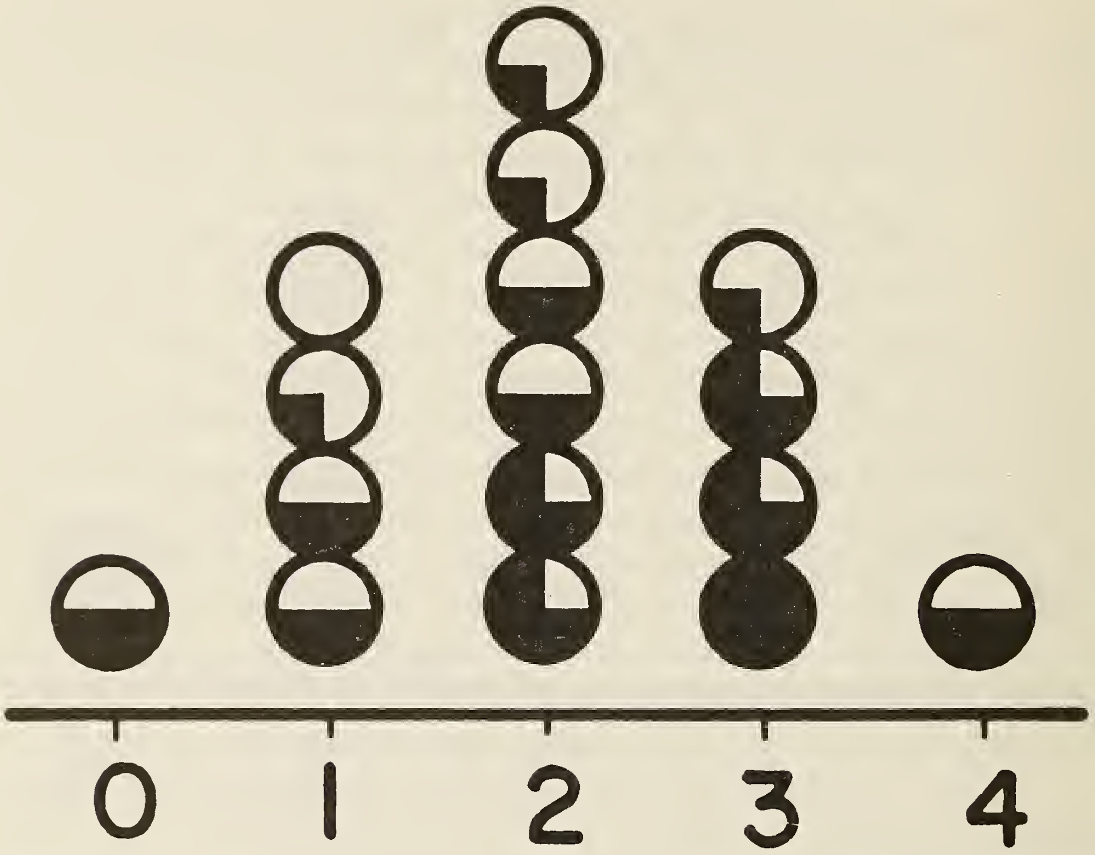
Figure 1. A Simplified Schema Relating Genotype to Phenotype When All Phenotypic Variance Is Attributable to Genetic Variation



Each circle represents an indivudal, and the percentage of all possible + alleles possessed by that individual is represented by the darkened area within the circle. The phenotypic dimension ranges from 0 through 4. This schema illustrates a situation in which heritability is 1.00; that is, there is a perfect correlation between genotype and phenotype.

have a higher phenotypic mean than did the population from which their parents were drawn. A similar operation in the case of a phenotype of lower heritability, as shown in figure 5, would provide a group of parents for which the number of + alleles that can be transmitted is considerably fewer. It is intuitively apparent that the offspring in the first example will inherit more + alleles than will the offspring in the latter example, and that the former offspring will have higher phenotypic values in the first selected generation than will those in the second example. Nonetheless, as long as heritability is nonzero, the selection procedure can be expected to yield offspring having a higher mean phenotypic value than that of the population from which their parents were drawn. Repeating the procedure in successive generations will have a cumulative effect, and the mean scores of the line from one generation to the next will gradually and

Figure 2. A Simplified Schema Relating Genotype to Phenotype When Some Phenotypic Variance Is Not Attributableto Genetic Variation

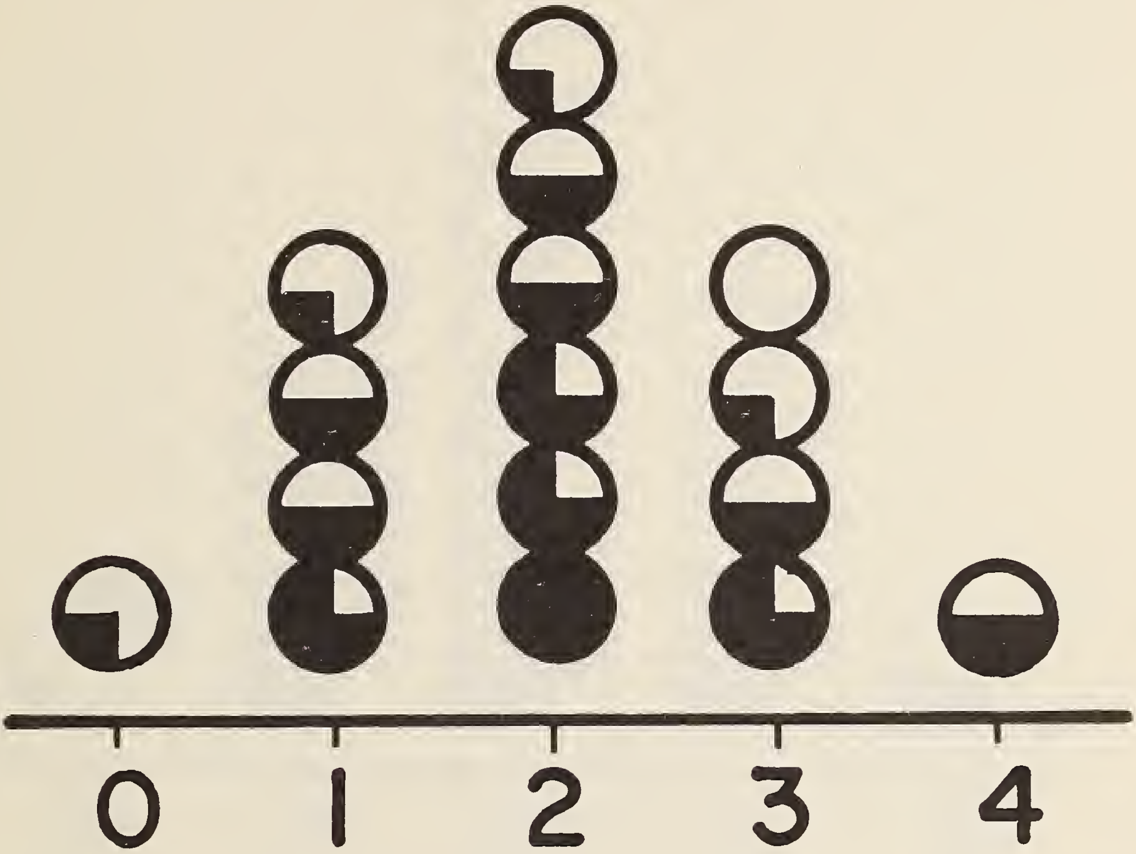


Nonzero heritability is indicated by a general tendency for individuals with greater percentages of all possible + alleles to fall toward the right end of a phenotypic dimension. This schema illustrates a situation in which heritability is less than 1.00, as indicated by the lack of a perfect correlation between genotype and phenotype.

systematically deviate more and more from the original population's average value.

In strictly practical agricultural applications, breeders may be interested only in selection in one direction—that which improves yield in one fashion or another. In basic research, however, one is usually interested in both extremes of a particular trait, and selection is performed in both an "upward" and a "downward" direction. Bidirectional selection of this sort has been performed successfully for a variety of phenotypes, and lessons can be learned from all of these selection studies for our special purposes of selecting for alcohol- or other drug-related parameters. However, because of the behavioral components of many of the alcohol-related phenotypes for which selection has been undertaken to date, it is particularly

Figure 3. A Simplified Schema Relating Genotype to Phenotype When Most Phenotypic Variance Is Not Attributable to Genetic Variation

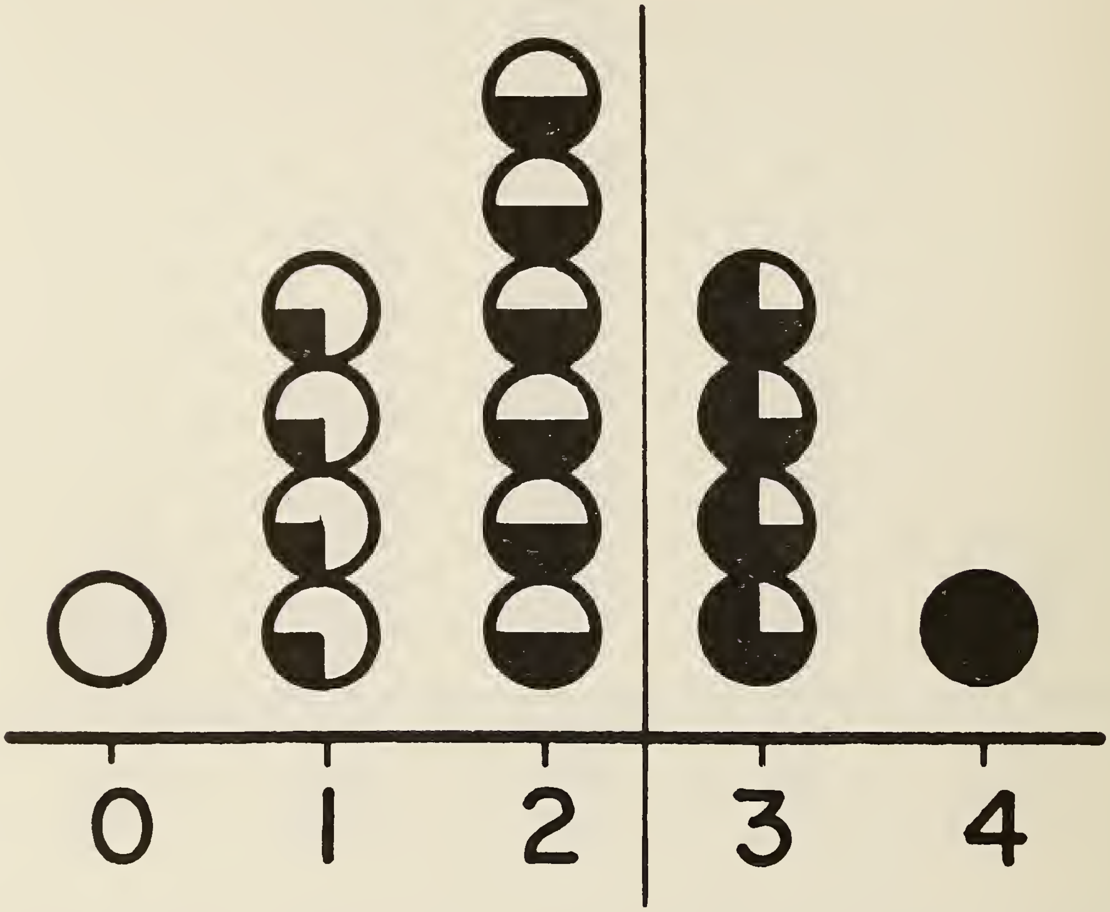


This schema illustrates a situation in which heritability is very low; that is, there is little systematic relationship between genotype and phenotype.

relevant that they should be viewed against a background of selection studies for other behavioral traits.

In his chapter DeFries provides a particularly good example when, for the purpose of illustrating basic principles, he describes his own research on locomotor activity. This is a landmark research program, and, as we shall see later, it incorporates all of the desiderata from the point of view of genetic design considerations. Hyde reviews the history of behavioral selection studies and surveys the contemporary scene. She shows that the early work in this area was handicapped by features that we now know are undesirable: e.g., foundation stocks were of uncertain heterogeneity (but certainly of *less* heterogeneity than could have been arranged); inbreeding was practiced (in some cases deliberately and in others by accidental default of small

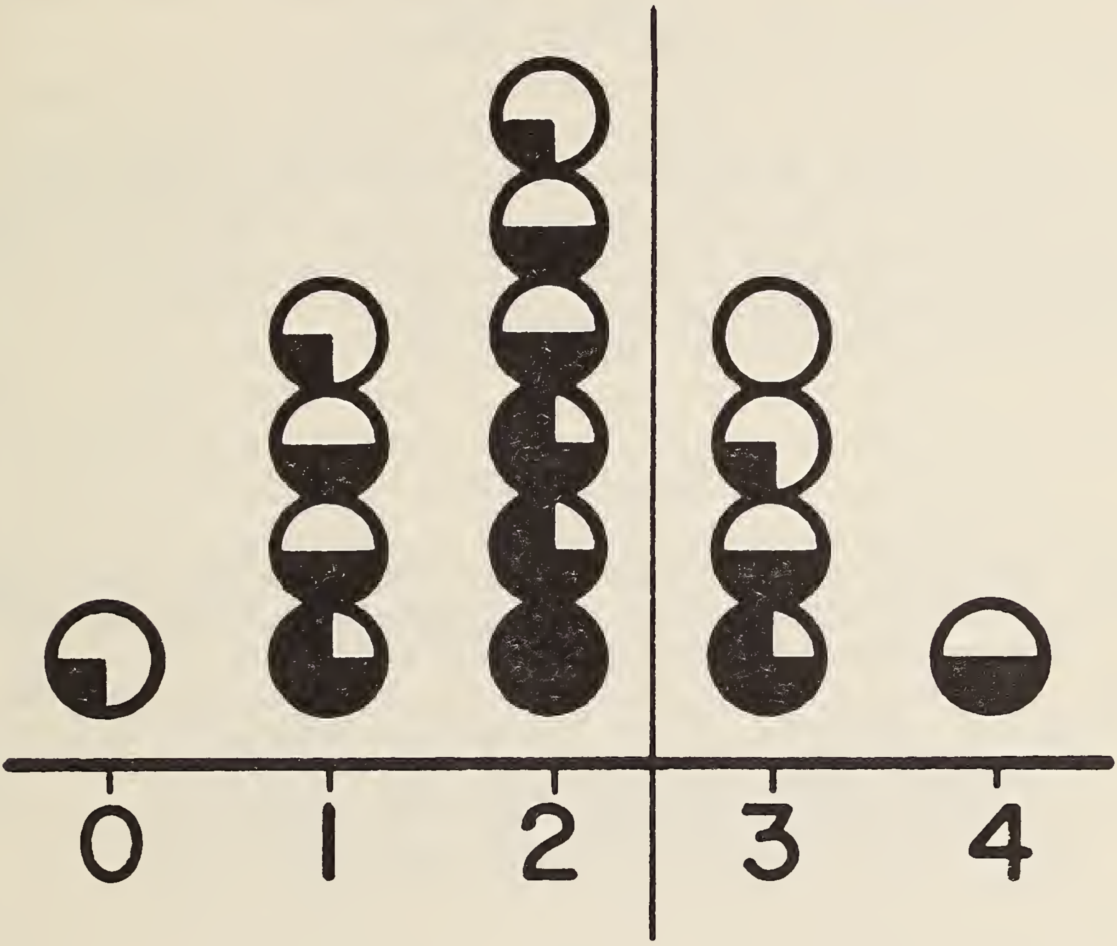
Figure 4. Genotypes of Phenotypically Selected Individuals When Heritability Is High



samples); in one case at least, the lines were not closed and matings were made across lines.

We should not be too critical of the shortcomings of these early research programs. At the time many of them were initiated, the features of polygenic theory were just emerging from the pens of the specialists. Fisher's classic work generalizing Mendelian rules to the polygenic situation was published in 1918. His book *The Genetical Theory of Natural Selection* was not published until 1930. The classic works of Haldane and Wright began appearing in the early 1920s and continued to appear throughout the following decade. Given the built-in time lag between initiation of selection studies and publication of their results, it is easy to see why the earlier works, dated in

Figure 5. Genotypes of Phenotypically Selected Individuals When Heritability Is Low



the 1920s and 1930s, were not optimally designed by current standards.

In the midyears of selection studies described by Hyde, the principal features of polygenic theory had, of course, already emerged. However, those whose primary training was not in quantitative genetics found these studies rather difficult to access. Symbology was not standardized, key papers were scattered throughout many years and many diverse journals, and many papers were too esoteric mathematically to be translated easily. Thus, many of those behavioral scientists who turned to selection procedures during the rapid growth phase of behavioral genetics (beginning around 1950) did so with a theoretical perspective that was spotty.

It is really quite astonishing that these early studies, with all their handicaps, were as successful as they were in generating large divergences between upward and downward selected lines. We might reasonably expect even more spectacular line differences with the more sophisticated and informed procedures applied in the recent behavioral selection studies that Hyde identifies.

These more recent and more satisfactory studies have, of course, had the opportunity to be informed by the dramatic developments in quantitative genetic theory that have occurred since the classic works of the pioneers in the field. The current status of this theoretical structure as it pertains to selective breeding is outlined by R.C. Roberts in his usual lucid and engaging manner.

References

Falconer, D.S. *Introduction to Quantitative Genetics.* New York: Ronald Press, 1960.

Fisher, R.A. The correlation between relatives on the supposition of Mendelian inheritance. *Trans R Soc Edinburgh*, 52:399-433, 1918.

Fisher, R.A. *The Genetical Theory of Natural Selection.* Oxford: Clarendon Press, 1930.

McClearn, G.E., and DeFries, J.C. *An Introduction to Behavioral Genetics.* San Francisco: Freeman, 1973.

Roberts, R.C. Some concepts and methods in quantitative genetics. In: Hirsch, J., ed. *Behavior-Genetic Analysis.* New York: McGraw-Hill, 1967. pp. 214-257.

Current Perspectives on Selective Breeding: Example and Theory

J.C. DeFries

Although various strategies for generating animal models by selective breeding are currently available, some selection designs are more efficient than others. Predicting the response to alternative selection schemes is the province of quantitative genetics (Falconer 1960; Pollak et al. 1977). Unfortunately, quantitative genetic theory often seems rather abstract, especially to investigators primarily concerned with mechanisms. In order to make the present discussion of selective breeding more concrete, this chapter shall begin with an example. It is hoped that the statistical models discussed in the latter part of the chapter will appear less abstruse after the results of the selection experiment are considered.

Genetics of Open-Field Behavior

The selection experiment to be discussed concerns "open-field" behavior in laboratory mice. The so-called "open-field test" consists of placing an animal, usually a rodent, in a brightly illuminated enclosure and scoring its behavior. Animals that obtain relatively low activity and high elimination (defecation and/or urination) scores when placed in this presumably stressful situation are regarded as being "emotional" or "reactive," whereas those with relatively high activity and low elimination scores are considered to be "nonemotional" or "nonreactive." Although the validity of the open-field test as an index of emotionality is controversial (Archer 1975; Broadhurst 1976), this test has been widely used as a prototype for the genetic analysis of complex behavioral characters.

Preliminary Analysis

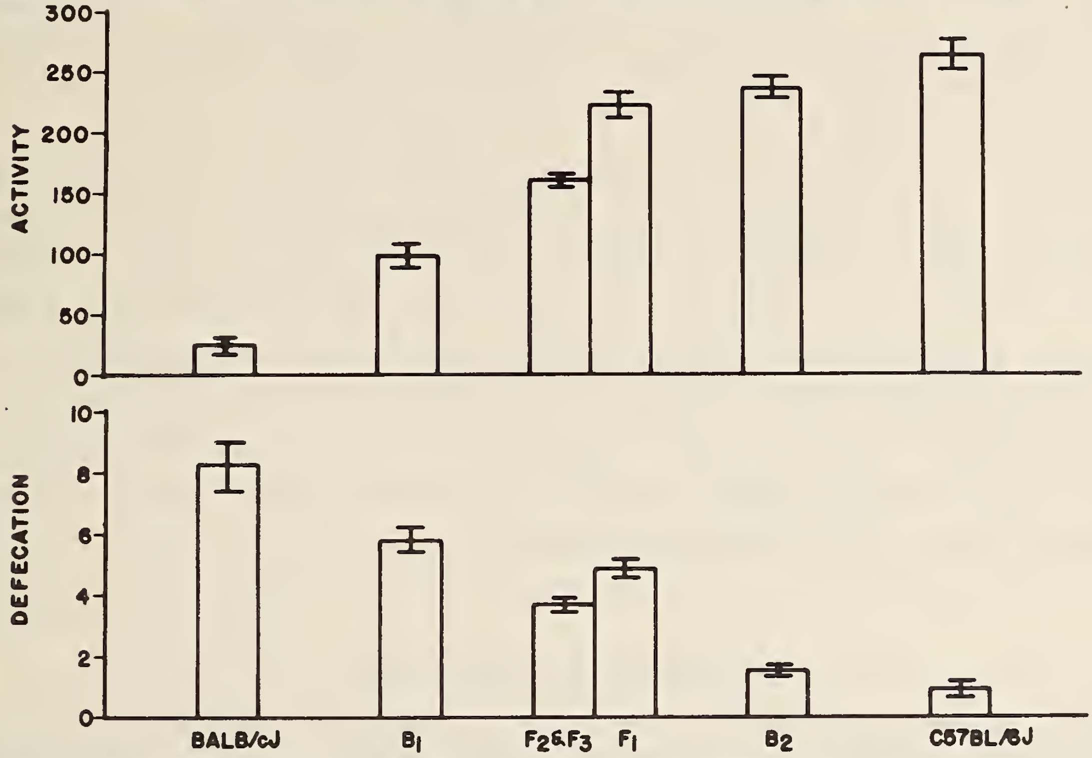
Before the selection experiment began, open-field behavioral data were obtained on 2,641 mice that were members of two highly inbred strains (BALB/cJ and C57BL/6J) and their derived F_1, backcross, F_2, and F_3 generations. Mice were tested individually for 3 minutes on each of 2 successive days at 40 ± 5 days of age in a 3-foot square automated open field. The field was constructed of white painted Plexiglas and was illuminated (approximately 48 footcandles of incident light) from above by two 20-watt fluorescent tubes. In order to automate the scoring of activity level, two sets of light sources were located on adjacent sides of the field and beamed through five equally spaced holes and infrared filters to photoconductive cells on the opposite sides. This arrangement provided a grid of light beams that partitioned the floor of the field in 36, 6 in. x 6 in. squares. Interruption of these light beams activated counters that recorded activity scores.

At the beginning of each test, a mouse was placed in a Plexiglas cylinder in one corner of the field. The mouse was released, a button was pushed to activate a timer, and the mouse was allowed to do whatever it wanted for 3 minutes. At the conclusion of the test period, the system was automatically deactivated, the mouse was removed, and the number of light beams interrupted and the number of fecal boluses deposited were recorded. Between tests, the floor of the field was rinsed with tap water and wiped dry with a paper towel.

The total number of light beams interrupted during the two test periods was used as each animal's activity score, and the total number of fecal boluses deposited was used as the defecation measure. Based upon the correlation (r) between day 1 and day 2 scores, the estimated reliabilities for the 2-day totals were about 0.8 and 0.5 for activity and defecation, respectively: reliability $= 2\,r/(1 + r)$. This calculation assumes that the same behaviors are being expressed on the 2 days, which may not be the case for open-field behavior. Nevertheless, these results suggest that reliabilities may sometimes be rather low, even for relatively simple measures such as open-field defecation. Fortunately, activity level was the major focus of the present selection experiment.

Average activity and defecation scores (2-day totals) for the two inbred strains and derived generations are summarized in figure 1. Because individual members of a given inbred strain are genetically identical, for all practical purposes, whereas members of different inbred strains may differ at many gene loci, a comparison of different

Figure 1.Mean Open-Field Activity and Defecation Scores



Average scores (± twice the standard error) are shown for BALB/cJ and C57BL/6 mice and their derived F_1, backcross (B_1 and B_2), F_2 and F_3 generations. Sample sizes were as follows: BALB = 64; B_1 = 257; F_2 and F_3 = 1,478; F_1 = 415; B_2 = 315; C57BL = 112.

Reproduced with the publisher's permission from DeFries et al. (1978). Copyright 1978 by Plenum Press.

inbred strains reared in the same laboratory environment provides prima facie evidence for the existence of heritable differences. From figure 1, it may be seen that there is about a 10-fold difference between the mean activity scores of the two inbred strains. The C57BL mice have an average activity score of 250, while members of the BALB strain have an average score of 25. Among the derived generations, there is a positive relationship between activity level and percentage of genes obtained from the C57BL strain. From left to right in this figure, these percentages are 0 (BALB), 25 (B_1), 50 (F_1, F_2, and F_3), 75 (B_2), and 100 (C57BL). It may also be seen in figure 1 that there is about an 8-fold difference between the mean defecation scores of the two inbred strains and a pronounced negative correlation with mean activity level across the various genetic groups.

Quantitative genetic analyses of these data (DeFries and Hegmann 1970) revealed that open-field activity and defecation scores are only moderately heritable characters. Narrow-sense heritability, which will be defined explicitly later in this chapter, was found to be only about 0.25 and 0.10 for activity and defecation, respectively. Nevertheless, evidence was obtained for the existence of a large negative genetic correlation, approximately -0.80, between the two characters. This finding of a large genetic correlation suggests that individual differences in the two characters are influenced by many of the same genes.

Minimum estimates of the number of loci responsible for differences in open-field activity and defecation scores between the two inbred strains were also obtained. These estimates were 3+ for activity and 7+ for defecation. Because these are minimum estimates, it was concluded that both characters are probably polygenic, i.e., influenced by genes at many loci. Nevertheless, evidence for a major gene effect was also obtained (DeFries et al. 1966). Albino mice, on the average, were found to have lower activity and higher defecation scores than pigmented mice.

Selection Experiment

Members of the F_3 generation were used as the foundation population for the selection experiment. Forty F_3 litters containing at least two males and two females were chosen at random. From 10 of these litters, the most active male and the most active female were selected to become progenitors of one high-activity line, H_1. From these same 10 litters, the least active male and the least active female were selected as progenitors for a low-activity line, L_1. From a second randomly chosen group of 10 F_3 litters, the same procedure was used to select progenitors for replicate selected lines, $H_2$and L_2. A male and female were chosen at random from each of 10 other F_3 litters to serve as progenitors for one control line (C_1). In the same manner, the progenitors of the second control line (C_2) were chosen from the remaining 10 F_3 litters. High-activity, low-activity, and control mice were then mated at random within line, yielding offspring representing the first selected generation (S_1).

In subsequent generations, within-litter selection was continued within the six closed lines. In other words, the most active male and female were selected from each litter within H_1 and H_2, the least active male and female were retained from each litter within L_1 and L_2, and a male and a female were chosen at random from each litter in C_1 and C_2. Animals retained for breeding were then mated at random within line. Thus, this design incorporated bidirectional, replicated within-litter selection and contemporaneous controls.

Because inbreeding reduces genetic variance within a selected line and the response to selection is a function of the genetic variance, it is important to minimize inbreeding during the course of a selection experiment. With 10 mating pairs or less in each of the six closed lines and within-litter selection, the expected increase in the coefficient of inbreeding in this experiment is less than 1.5 percent per generation.

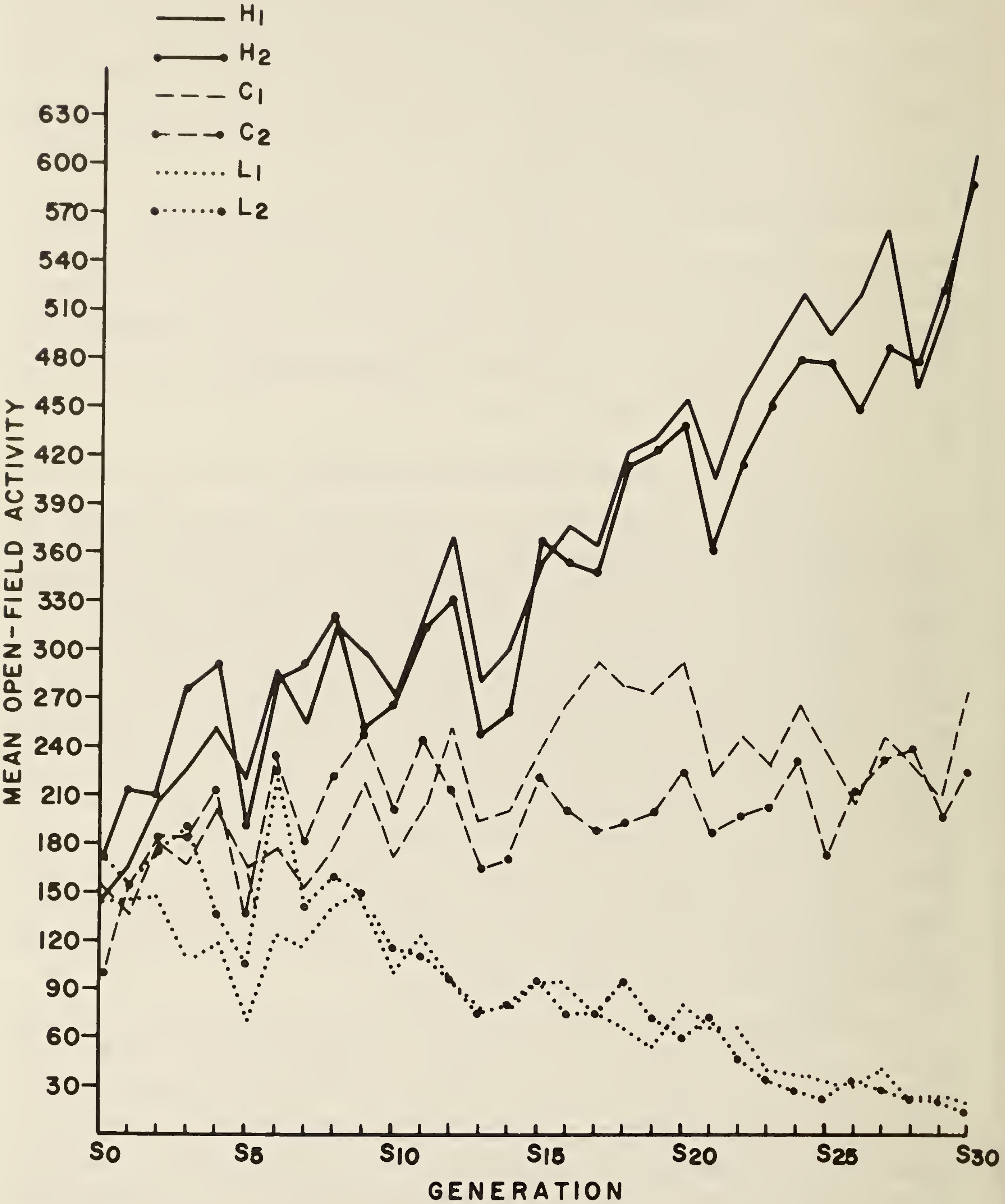
Thirty generations of selection were completed between June 1966 and November 1976. The number of mice tested in the six lines each generation varied from 44 to 108. The total number of mice tested in generations S_1 through S_{30} was 14,184.

From figure 2, it may be seen that a marked and highly reliable response to selection was achieved. The mean activity scores of the two high-activity lines are more than 30 times higher than those of the low-activity lines after 30 generations of selection. The generality of this direct response is indicated by the similarity of the mean activity scores of the replicate lines, namely, H_1 versus H_2, C_1 versus C_2, and L_1 versus L_2.

A comparison of figures 1 and 2 shows that the difference between the high-activity and low-activity lines exceeds that observed between the two inbred parental strains (BALB and C57BL) by about a factor of 3. How could this have happened? If members of the C57BL strain had been carrying all of the genes for high activity and members of the BALB strain had only genes for low activity, it would have been impossible for recombination and subsequent selection to have yielded a difference that exceeded that between the two inbred strains. The fact that this difference was exceeded indicates that members of the C57BL strain must have been homozygous for some genes for low activity and that members of the BALB strain must have been homozygous for some genes for high activity. The magnitude of the response, plus the observation that the response to selection continued for 30 generations, supports the previous evidence for the polygenic nature of this behavior.

The direct response to selection was relatively symmetrical through about S_{20}, i.e., the difference between the high-activity lines and the control lines was approximately the same as that between the control lines and low-activity lines. During the latter part of the experiment, however, an asymmetrical response occurred. The lesser response by the low-activity lines was apparently the result of a “floor effect,” whose existence is obvious in figure 3. The distribution of the activity scores in the foundation population is depicted at the top of this figure, and the distributions for the high and low lines (pooled across replicates) are shown below at five-generation intervals. The mean of the two control lines is indicated by an arrow. It may be seen that there is a marked “piling up” of scores at the lower end of the

Figure 2. Mean Open-field Activity Scores



Average activity scores are shown for six lines of mice: two selected for high open-field activity (H_1 and H_2), two selected for low open-field activity (L_1 and L_2), and two randomly mated within line to serve as controls (C_1 and C_2).

Reproduced with the publisher's permission from DeFries et al. (1978). Copyright 1978 by Plenum Press.

distribution in the foundation population and that this becomes more pronounced in the low lines during the course of the selection experiment. Nevertheless, the distributions of the high and low lines are completely nonoverlapping by S_{30}. The most active mouse in the low lines is considerably less active than the least active mouse in the high lines. This complete separation of the distributions of the high- and low-activity lines vividly demonstrates the power of selection to modify a character, even one with a moderately low heritability.

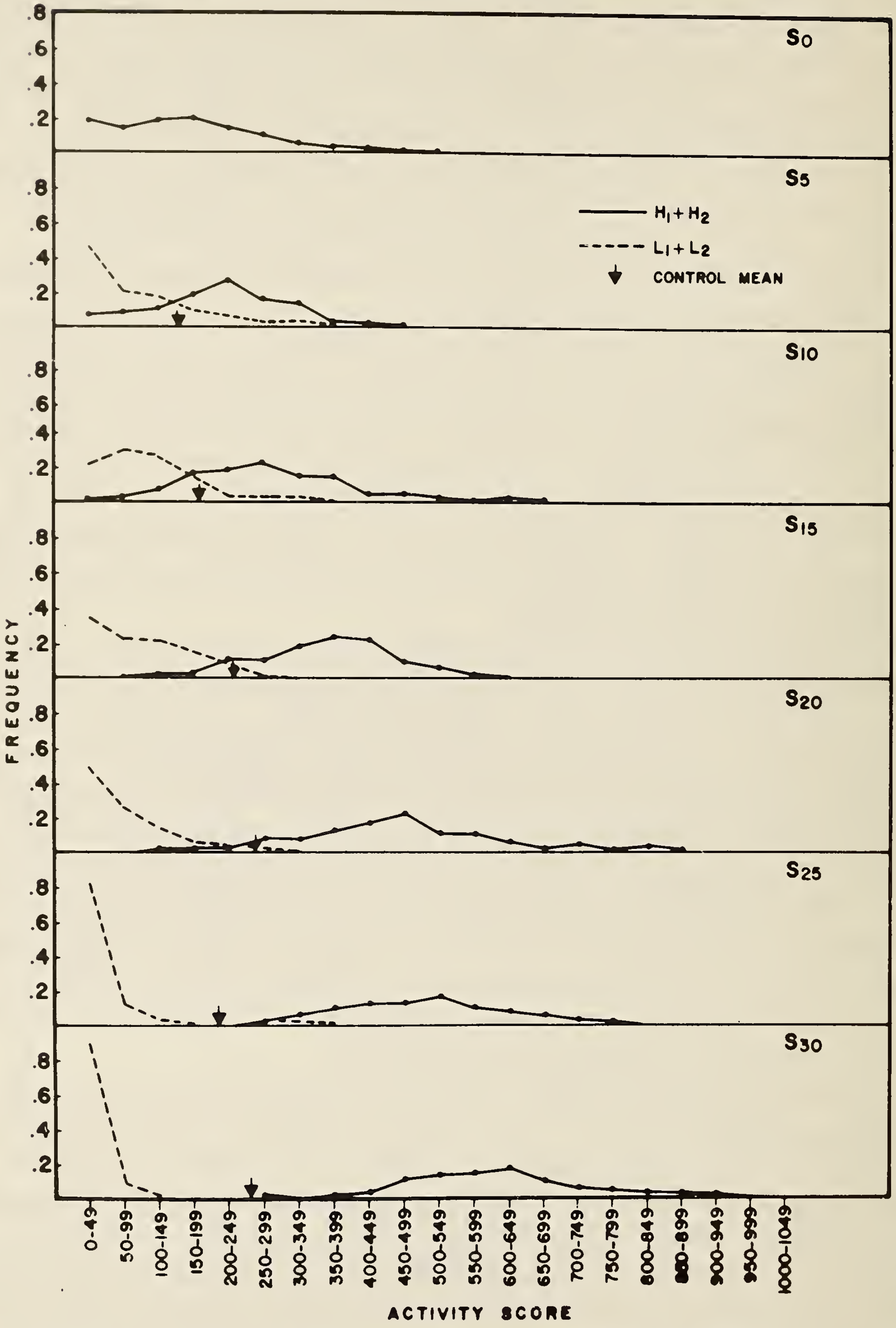
Corresponding distributions for the defecation scores of the high- and low-activity lines are presented in figure 4. Although there is again evidence for a floor effect, the distributions of the defecation scores also diverge as a function of selection for open-field activity. In S_{30}, the mean open-field defecation score of the low-activity lines is about seven times higher than that of the high-activity lines and there is relatively little overlap in their distributions. This is a correlated response to selection, and it again demonstrates the existence of a large, negative genetic correlation between open-field activity and defecation scores.

It was previously stated that albino mice, on the average, obtain lower activity and higher defecation scores than pigmented mice. If this is indeed the case, then selection for low open-field activity should result in an increase in the frequency of albinism and selection for high open-field activity should be accompanied by a decrease in the frequency of albinism. It may be seen in figure 5 that this is exactly what happened. The frequency of albinism, which is about 0.25 in the foundation population, increased in both L_1 and L_2, but decreased in H_1 and H_2 as a function of selection. In fact, the albino allele became fixed in L_2 (100 percent albino) during S_8 and in L_1 during S_{23}. In contrast, this allele was apparently eliminated in H_2 and was at a very low frequency in H_1 during S_{30}.

It may also be seen in figure 5 that a divergence in the frequency of albinism occurred between the replicate control lines. Because no artificial selection was applied to these lines, this divergence is apparently due to chance changes in gene frequency, i.e., "random genetic drift." Such chance effects should be more important for single-locus characters such as albinism than for polygenic characters such as open-field activity. Recall that the mean activity scores of C_1 and C_2 (see figure 2) were rather similar during the course of the selection experiment.

The divergence between C_1 and C_2 for the frequency of albinism clearly demonstrates the importance of maintaining replicated lines in a selection experiment. If only C_1 had been maintained, the

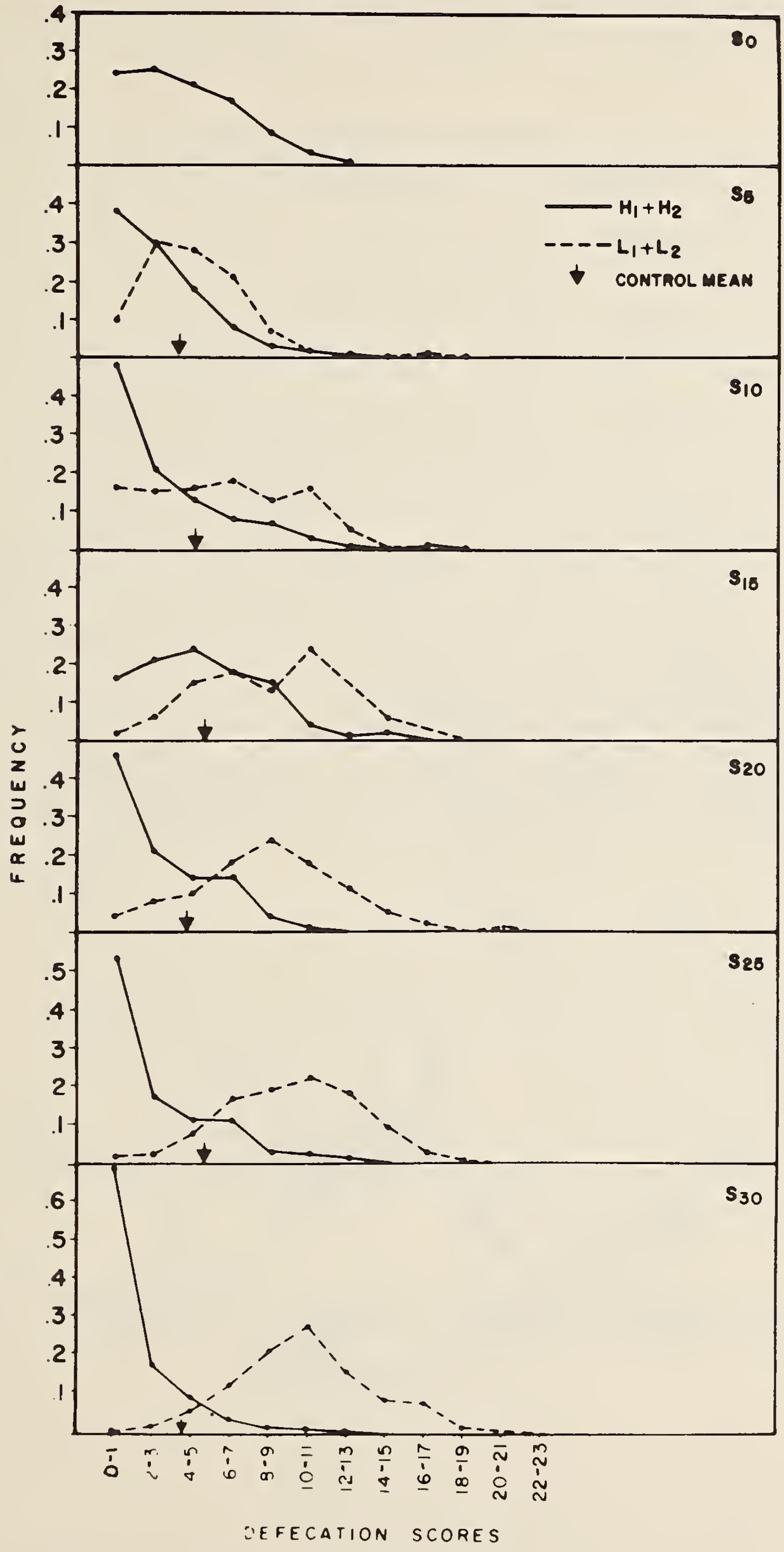
Figure 3. Activity Score Distributions



Distributions of activity scores of lines selected for high and low open-field activity are depicted. Average activity of controls in each generation is indicated by an arrow.

Reproduced with the publisher's permission from DeFries et al. (1978). Copyright 1978 by Plenum Press.

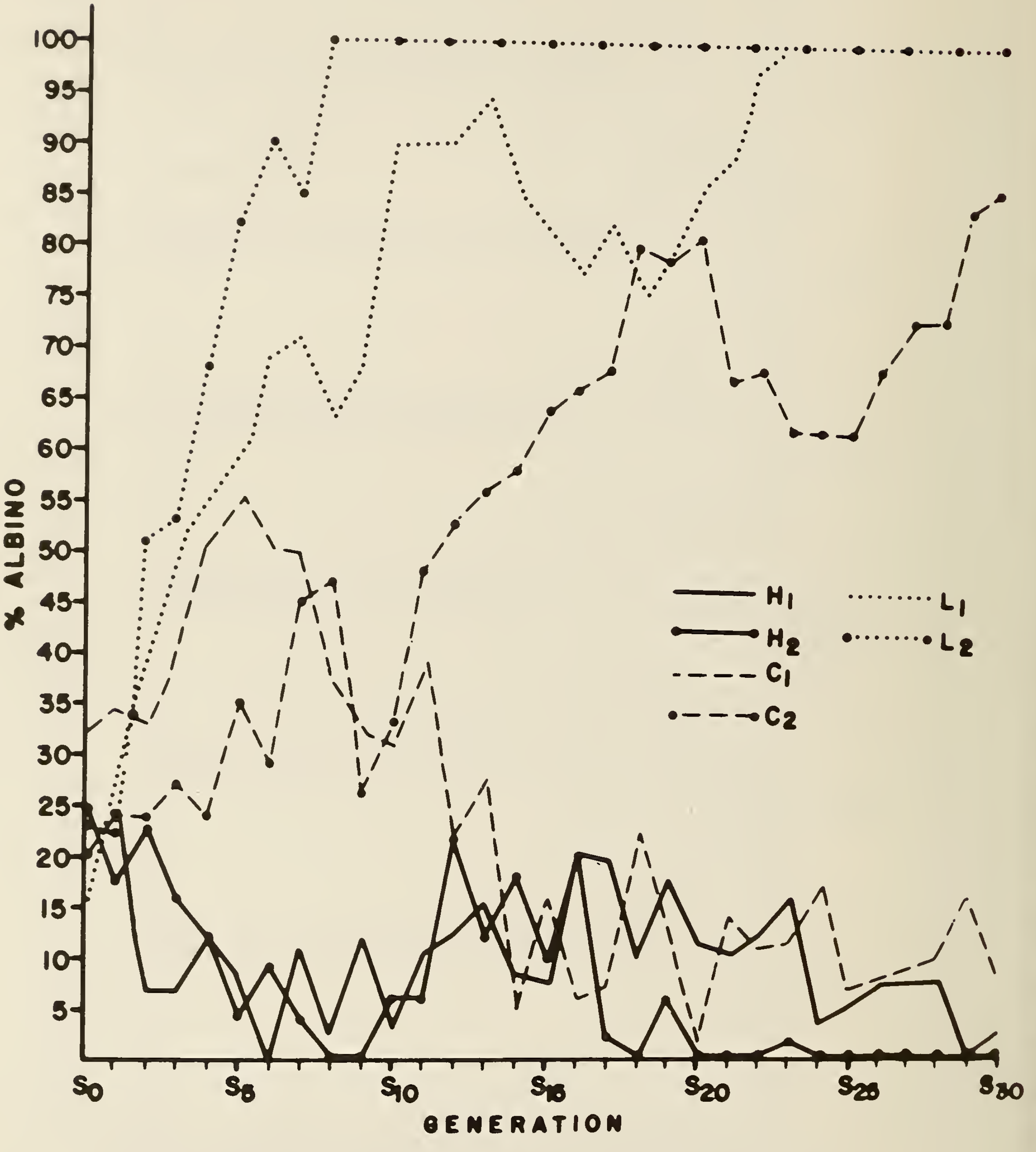
Figure 4. Defecation Score Distributions



Distributions of defecation scores of lines selected for high and low open-field activity are shown. Average defecation score of controls in each generation is indicated by an arrow.

Reproduced with the publisher's permission from DeFries et al. (1978). Copyright 1978 by Plenum Press.

Figure 5. Frequency of Albinism



Percentages of albino mice are shown for the six lines: two selected for high open-field activity (H_1 and H_2), two selected for low open-field activity (L_1 and L_2), and two randomly mated within line to serve as controls (C_1 and C_2).

Reproduced with the publisher's permission from DeFries et al. (1978). Copyright 1978 by Plenum Press.

decrease in the frequency of albinism in that line would likely have been interpreted as being due to natural selection against albinism. The increase in frequency of albinism in C_2 renders that hypothesis untenable.

Because random genetic drift may also occur in selected lines, a correlated response in an unreplicated selection experiment must be interpreted with caution. For example, assume that selection has been performed for some behavioral character (X) and that one high line and one low line have been established. After separation between the lines has occurred for character X, we wish to test the hypothesis that this difference in behavior is due to a difference in the activity level of some enzyme (character Y). Assay of the two lines results in a pattern of results summarized in table 1. Although these results are consistent with the hypothesis, they are by no means definitive proof of a causal relationship. As in the case of albinism in C_1 and C_2, random genetic drift could have caused the difference between the two lines in character Y; thus, the apparent associations between X and Y could be purely fortuitous. However, if the lines had been replicated and the pattern of results depicted in table 2 had been obtained, the likelihood that the association was fortuitous would be greatly reduced.

Table 1. Correlated Response to Selection in Unreplicated Lines

	Character	
Line	Selected(X)	Correlated(Y)
High	+	+
Low	–	–

Reproduced with publisher's permission from DeFries(1980). Copyright 1980 by Boxwood Press.

Table 2. Correlated Response to Selection in Replicated Lines and Controls

Line	Character	
	Selected(X)	Correlated(Y)
H_1	+	+
H_2	+	+
C_1	0	0
C_2	0	0
L_1	–	–
L_2	–	–

Reproduced with the publisher's permission from De Fries (1980). Copyright 1980 by Boxwood Press.

In fact, the lines selected for open-field activity have recently been assayed for a brain enzyme, glutamic acid decarboxylase (GAD), which catabolyzes glutamate to GABA, an inhibitory transmitter (George et al. 1980). In addition to GAD activity (both K_m and V_{max}), endogenous levels of GABA and glutamate were also determined. As predicted from results of several previous studies that utilized inbred rat or mouse strains (Gaitonde and Festing 1976; Rick et al. 1971; Tunnicliff et al. 1976), GABA production was found to be inversely correlated with open-field activity. However, none of the measured components of the GABA system accounted for as much as 15 percent of the variance in open-field activity among lines. Moreover, the rank order of the lines was not as predicted for a genetically correlated character, and the magnitude of the differences among lines in enzyme activity was not large. Thus, it was concluded that the GABA system is not an important mediator of individual differences in open-field behavior.

Theory

How do quantitative geneticists explain the results of a selection experiment? A very brief overview of quantitative genetic theory will help in our attempt to answer this question. (Those interested in more detail may refer to Falconer 1960 or to McClearn and DeFries 1973.)

The Model

Quantitative genetic theory begins with the entirely reasonable assumption that an observed character measured on an individual (i.e., the phenotype) is a function of both the individual's genotype and the environmental circumstances in which it developed:

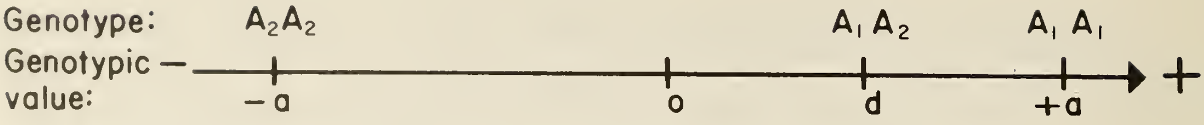
$$P = f(G,E), \tag{1}$$

where P is the phenotypic value (the value for some character measured on an individual, usually expressed as a deviation from the population mean), G is a corresponding genotypic value (the value conferred upon the individual by its genotype, also expressed in metric units), and E is an environmental deviation due to all sources of nongenetic influence. Before we consider the nature of this relationship, let us first explore the meaning of G in more detail.

We shall begin with the simplest case: two alleles (alternate forms of a gene) at a single, autosomal locus. For this case, three different genotypes may occur (A_1A_1, A_1A_2, and A_2A_2). Let A_1A_1 symbolize the homozygote with higher value, i.e., the genotype that is homozygous for the "increasing" allele. Values may then be arbitrarily assigned to the different genotypes as depicted in figure 6. The point that bisects the difference between the two homozygotes may be defined as the origin, and the genotypic values may be expressed as deviations from this midhomozygote point. A_1A_1 and A_2A_2 deviate from this point by the same amount; thus, their assigned genotypic values are $+a$ and $-a$, respectively. The genotypic value for A_1A_2, symbolized d, will depend upon the gene action present at that locus. For example, if A_1 is dominant to A_2, d will be positive. However, if A_2 is dominant to A_1, d will be negative. If there is complete dominance, d will equal either $+a$ or $-a$. If there is overdominance, d will be greater than $+a$ or less than $-a$. On the other hand, if there is no dominance, d will equal zero.

As an example, let us again consider the influence of albinism on a measure of open-field behavior. In the F_2 and F_3 generations derived from a cross of two inbred strains (BALB/cJ and C57BL/6J), the mean transformed (square root) activity scores of pigmented and albino mice were approximately 12 and 10, respectively (DeFries et al. 1966). Because the difference between the mean scores of homozygous pigmented (CC) and albino (cc) mice is equal to $2a$, then

Figure 6. Assigned Genotypic Values



Adapted from *Introduction to Quantitative Genetics*, p. 113, by D.S. Falconer (1960). Copyright 1960 by Longman Group Limited.

$a = (12 - 10)/2 = 1$. Assuming complete dominance of the C allele for high open-field activity, d is also equal to 1.

The C57BL inbred strain is pigmented (CC) , whereas the BALB strain is albino (cc). Thus, the frequency of the c allele in the F_2 and F_3 generations was approximately one-half. In these segregating, unselected generations, the following genotypic distribution should have occurred:

$$1/4\ CC + 1/2\ Cc + 1/4\ cc. \tag{2}$$

From this distribution, we may calculate the mean genotypic value (M) in the foundation population as follows:

$$M = 1/4\ (a) + 1/2\ (d) + 1/4\ (-a)$$

$$= 1/4\ (+1) + 1/2\ (+1) + 1/4\ (-1) = 1/2, \tag{3}$$

where M is expressed as a deviation from the midhomozygote point.

Selection for high open-field activity increased the frequency of the C allele and thereby increased in the mean genotypic value. In contrast, selection for low open-field activity decreased the frequency of the C allele (increased the frequency of c) and thus decreased M . Recall, however, that open-field activity is influenced by genes at many loci, not just by alleles at the c locus.

For any random mating population, genotypic frequencies may be predicted from gene frequencies (McClearn and DeFries 1973) as follows:

$$(pA_1 + qA_2)^2 = p^2A_1A_1 + 2pqA_1A_2 + q^2A_2A_2, \tag{4}$$

where p and q are the frequencies of A_1 and A_2 in the population. When $p = q = 1/2$, as in the case of albinism in the F_2 and F_3 generations of the preliminary genetic analysis, the genotypic proportions are 1/4, 1/2, and 1/4.

The three genotypes, their frequencies, and their genotypic values are summarized in table 3. From this table, the mean genotypic value for the more general case is obtained as follows:

$$M = p^2(+ a) + 2pq(d) + q^2(- a) = a(p^2 - q^2) + 2\ pqd$$

$$= a(p + q)(p - q) + 2pqd = a(p - q) + 2\ pqd \tag{5}$$

since $p + q = 1$.

Table 3. Genotypic Value and Gene Dosage

Genotype	Frequency	Genotypic Value	Gene Dosage [$N (A_1)$]
$A_1 A_1$	p^2	a	2
$A_1 A_2$	$2pq$	d	1
$A_2 A_2$	q^2	$-a$	0

Reproduced from *Introduction to Behavioral Genetics* by G.E. McClearn and J.C. DeFries (1973). Copyright 1973 by W.H. Freeman and Company.

From this expression for M , it may be seen that the mean genotypic value in a population depends upon both the genotypic values, a and d , and the gene frequencies, p and q. Selection for lower values decreases p , increases q , and results in a corresponding decrease in M. In a nutshell, that is the way in which selective breeding changes a character. Selection changes the frequencies of genes in a population, and M changes accordingly.

The genotypic value reflects the joint effect of alleles in combination with other alleles. However, genes, not genotypes, are transmitted from one generation to the next. The resemblance of relatives and the response to selection are functions of the average effects of the genes. The average effect of A_1 and A_2, symbolized α_1 and α_2, may be expressed as a function of the genotypic values and gene frequencies (Falconer 1960) as follows:

$$\alpha_1 = q\left[a + d(q - p)\right]$$

$$a_2 = -p\left[a + d(q - p)\right] \tag{6}$$

The difference between α_1 and α_2, symbolized α, is referred to as the average effect of a gene substitution. It may be shown (McClearn and DeFries 1973) that

$$\alpha = a + d(q - p) = b_{\mathrm{GN(A1)}} \tag{7}$$

where $b_{\mathrm{GN(A1)}}$ is the regression of the genotypic value on the gene dosage, i.e., the average linear change in genotypic value corresponding to a unit change in the number of A_1 alleles in the genotype (table 3).

The average effects of the alleles may be summed to yield the additive genetic value, symbolized A. This summation is shown for the single-locus case in table 4. For the polygenic case, the average effects of the genes may be summed across all loci that influence the character, as well as across the alleles at each given locus. The additive genetic value is of considerable importance to selection theory because the expected genotypic value of an individual's progeny is equal to one-half of the parent's additive genetic value (McClearn and DeFries 1973).

Table 4. Additive Genetic Values for Single-Locus Case

Genotype	Additive Genetic Value(A)
$A_1 A_1$	$2\alpha_1 = 2q\alpha$
$A_1 A_2$	$\alpha_1 + \alpha_2 = (q-p)\alpha$
$A_2 A_2$	$2\alpha_2 = -2p\alpha$

Reproduced from *Introduction to Quantitative Genetics*, p.121, by D.S. Falconer. Copyright 1960 by Longman Group Limited.

When genotypic values and additive genetic values are both expressed as deviations from M, G will equal A only when $d = 0$, i.e., when there is no dominance. Thus, for the single-locus case:

$$G = A + D, \tag{8}$$

where D is a dominance deviation due to nonlinear interactions that may occur between alleles at the *same* locus. For the polygenic case, one more term must be added to the equation:

$$G = A + D + I, \tag{9}$$

where I refers to epistatic deviations (nonlinear interactions between alleles at *different* loci). To illustrate, consider genotypic values at two loci:

$$\begin{aligned} G_1 &= A_1 + D_1 \\ G_2 &= A_2 + D_2. \end{aligned} \tag{10}$$

When considering both genotypes jointly, however:

$$\begin{aligned} G &= G_1 + G_2 + I \\ &= A_1 + D_1 + A_2 + D_2 + I \\ &= A + D + I, \end{aligned} \tag{11}$$

where G is the genotypic value due to the joint effects of genes at both loci, A is the sum of the additive genetic values across loci, D is the sum of the dominance deviations, and I is due to any nonlinear interactions that may occur between alleles at the two loci. This partitioning of G into parts is an important aspect of quantitative genetic theory because A, D, and I contribute differentially to the resemblance of relatives and the response to selection (Falconer 1960).

Heritability

The observed phenotypic value for an individual may also be represented by a linear model:

$$P = G + E + (G \times E), \tag{12}$$

where $(G \times E)$ symbolizes a deviation due to any nonlinear interactions that may occur between G and E (Plomin et al. 1977). The left and right sides of this equation may be squared, summed across individuals in a population, and divided by N (recall that P is expressed as a deviation from the mean) to yield the various components of the phenotypic variance:

$$V_P = \frac{\Sigma P^2}{N} = \frac{\Sigma[G + E + (G \times E)]^2}{N}$$

$$= \frac{\Sigma G^2 + \Sigma E^2 + \Sigma(G \times E)^2 + \Sigma 2GE}{N}$$

$$= V_G + V_E + V_{G \times E} + 2Cov_{GE}, \tag{13}$$

where Cov_{GE} represents any covariance that may occur between genotypic values and environmental deviations (Plomin et al. 1977).

The genotypic variance may be partitioned in exactly the same manner to yield the following components:

$$V_G = V_A + V_D + V_I, \tag{14}$$

where V_A is the additive genetic variance, V_D is the dominance variance, and V_I is variance due to epistatic interactions. Because

A, D, and I are statistically independent, no covariance is necessary in this equation.

The concept of heritability is based upon this variance-partitioning procedure. Lush (1940) defined heritability as "the fraction of the observed variance that was caused by differences in heredity. This fraction is a statistic describing a particular population. It can be made larger or smaller if either the numerator or the other ingredients in the denominator can be altered. Thus it may vary from population to population for the same characteristic and may vary from one characteristic to another even in the same population" (Lush 1940, pp. 293-294).

Lush later (1949) distinguished between heritability in its "narrow sense" and heritability in its "broad sense":

$$\text{narrow-sense heritability} = V_A / V_P$$

$$\text{broad-sense heritability} = V_G / V_P. \tag{15}$$

These variance ratios may be symbolized h^2 and h_B^2, respectively. Methods for estimating heritability are discussed in some detail by Falconer (1960).

As defined above, heritability is obviously a *descriptive* statistic. It describes how much of the observed variation in a population is due to individual differences in genotypic or additive genetic values. However, heritability is also predictive. When G and E are uncorrelated (as is likely the case with most laboratory studies where the environment can be randomized across genotypes), h^2 may be shown to be equivalent to b_{AP}, the regression of the additive genetic value on the phenotypic value (Falconer 1960). Thus, from an estimate of h^2 and measured phenotypic values, additive genetic values may be predicted.

In addition, h^2 may be used to predict the performance of an individual's offspring. Because the expected genotypic value of the offspring of an individual is equal to one-half of the additive genetic value of that individual, the genotypic covariance of offspring and parent contains $1/2\,V_A$:

$$CovPO = CovGG_o = Cov(A + D)(1/2A) = 1/2V_A. \tag{16}$$

In the absence of a correlation between parent and offspring environmental influences, the regression of offspring on parent is as follows:

$$b_{op} = CovPO/V_P = 1/2V_A/V_P = 1/2h^2. \tag{17}$$

When both parents are measured, a midparent value (average of father's and mother's scores, symbolized $\overline{P}$) may be calculated. It may be shown that

$$Cov\overline{P}O = CovPO = 1/2V_A \text{ and } V_{\overline{P}} = 1/2V_P, \tag{18}$$

when mating is at random. Thus,

$$b_{o\bar{p}} = 1/2V_A/1/2V_P = h^2. \tag{19}$$

From the above expressions it may be seen that heritability may be used to predict parent-offspring resemblance and vice versa, i.e., heritability may be estimated from the similarity of parents and their offspring.

The concept of heritability is of paramount importance for the generation of animal models by selective breeding. All equations that are utilized to predict the response to selection for polygenic characters are functions of heritability, regardless of their complexity. In order to illustrate the simplest case, consider the following regression equation:

$$\hat{Y} = \overline{Y} + B_{yx}(X - \overline{X}). \tag{20}$$

Recall that if two variables (X and Y) are correlated, we may predict values of $Y(\hat{Y})$ from observed values of X. Now, let $\hat{Y} = P_o$ (expected phenotypic value of offspring) and X equal the midparent value, $\overline{P}$ Upon substitution,

$$\hat{P}_o = M + b_{op}(\overline{P} - M). \tag{21}$$

Alternatively,

$$\hat{P}_o - M = b_{op}(\overline{P} - M).$$

The symbol $\overline{P}$ represents the mean of the selected parents, $\overline{P} - M$ is the selection differential (symbolized S), and $P_o - M$ is the expected response to selection (R). We have already shown that $b_{op} = h^2$. Thus, for the case of "mass" selection, where each individual is judged solely on the basis of one measured value,

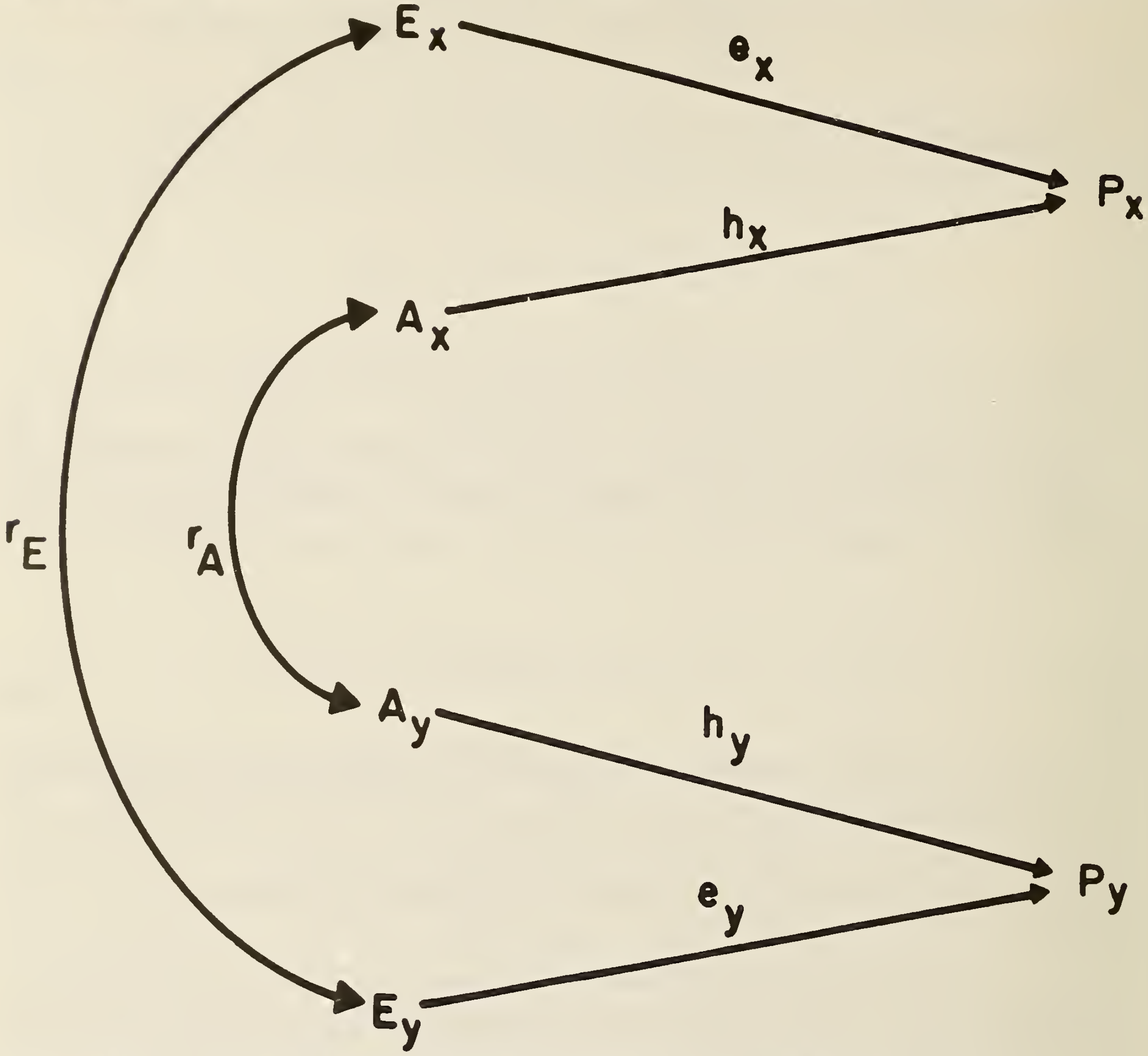
$$R = h^2 S. \tag{22}$$

As an example, assume a population in which each individual has been measured once for some character, X. If the mean phenotypic value in the population is 40, the mean for a group of individuals retained for breeding is 60, and the heritability of character X is 0.40, then $S = \overline{P} - M = 60 - 40 = 20$, and $R = h^2 S = (0.40)(20) = 8$. The expected change in the mean as a result of one generation of selection is 8; i.e., the score of the progeny of the selected individuals is expected to average 48, or 8 units above the mean of the previous (unselected) generation. By selecting more intensively, S —and thereby R —could be increased.

Genetic Correlation

When two or more characters are measured on each individual in a population, the phenotypic correlation between the characters may also be partitioned into genetic and environmental parts. The basic model is indicated in the path diagram depicted in figure 7. A path coefficient may be defined as the proportion of the standard deviation (σ) in a dependent variable due to variation in an independent variable. From the model of quantitative genetics, all of the variation in A and E is reflected in P. Thus, the path from A to P is σ_A/σ_P, the square root of h^2. Likewise, the path from E to P is σ_E/σ_P, the square root of $1 - h^2$. The correlation between A_x and A_y (r_A) is a genetic

Figure 7. Phenotypic Correlation Between Two Characters



Path diagram of the observed correlation between two characters (P_x and P_y) measured on an individual as a function of the genetic correction (r_A) and the environmental correlation (r_E).

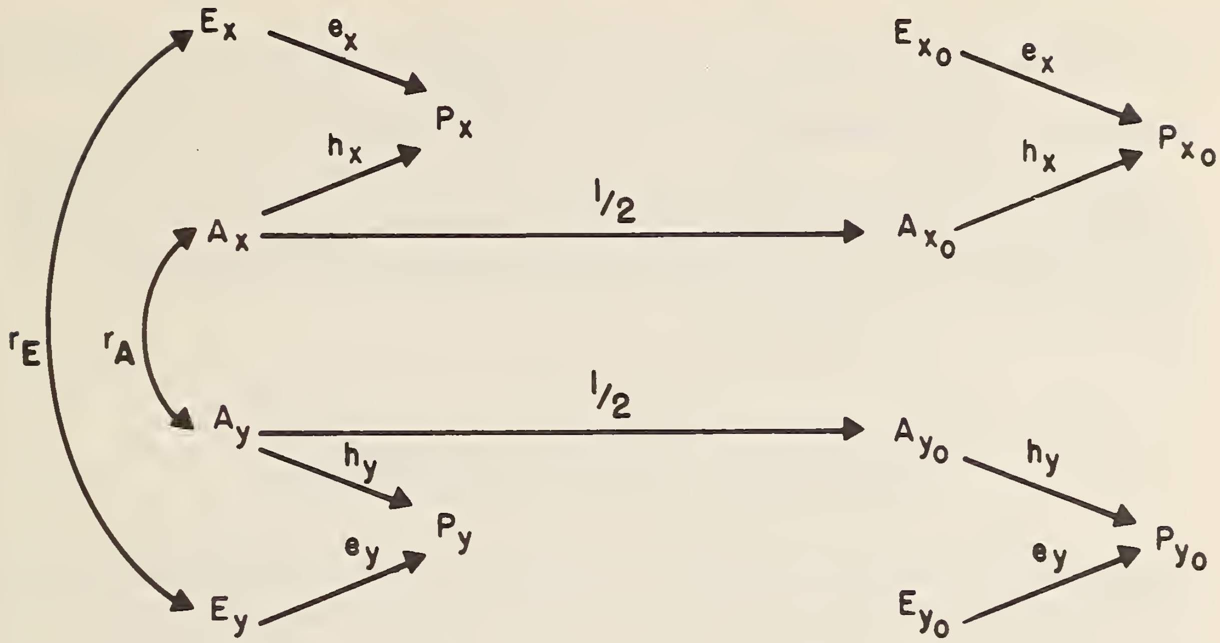
Reproduced with the publisher's permission from DeFries et al. (1979). Copyright 1979 by Sijthoff and Noordhoff.

correlation and that between E_x and E_y (r_E) is a corresponding environmental (plus nonadditive genetic) correlation. From the rules of path analysis, it may be shown that the phenotypic correlation between X and Y(r_P) is a function of r_A and r_E as follows:

$$r_P = h_x h_y r_A + e_x e_y r_E. \tag{23}$$

The concept of the genetic correlation is analogous to that of heritability. For example, whenever a data set is sufficient for estimating h^2, then r_A may be estimated from the same data set if

Figure 8. Phenotypic Cross Correlations



Path diagram of bivariate resemblance between parental phenotypes (P_x and P_y) and offspring phenotypes ($P_{x\,o}$ *and* $P_{y\,o}$)

Reproduced with publisher's permission from DeFries et al. (1979). Copyright 1979 by Sijthoff and Noordhoff.

more than one variable has been measured on each individual. Consider the path diagram of bivariate parent-offspring resemblance in figure 8. The expectation for the single-parent/single-offspring correlation for character *Y* is $1/2h_y^2$. In exactly the same manner, the parent-offspring correlation for character *X* equals $1/2h_x^2$. From this figure it may be seen that the cross-correlation between character *X* in the parent and character *Y* in the offspring (and vice versa) estimates $1/2\ h_x h_y r_A$. Thus, when two characters have been measured on a large sample of parents and their offspring, the heritabilities of both characters and the genetic correlation between them may be estimated.

When a large genetic correlation is found between two characters, as was the case between open-field activity and defecation in the example previously discussed, it may be concluded that both characters are probably influenced by many of the same genes. Thus, r_A is a descriptive statistic. However, just like h^2, r_A is also predictive. Selection for character *X* will result in a change in character *Y* as a function of their genetic correlation:

$$CR_y = r_A h_x h_y S_x \sigma_{P_y} / \sigma_{P_x}, \tag{24}$$

where CR_y is the correlated response in character Y.

Optimal selection designs for generating animal models involving more than one variable are based on the genetic correlations among the variables, as well as their individual heritabilities (see the next chapter by R.C. Roberts).

Summary and Conclusions

A selection experiment for open-field activity in laboratory mice was described to illustrate some of the practical considerations of a selective breeding program. The selection design was bidirectional, with replicated selected and control lines. After 30 generations of within-litter selection, the mean open-field activity scores of the two high lines exceeded those of the two low lines by a factor of 30 and their distributions were completely nonoverlapping. A correlated response to selection occurred for open-field defecation, substantiating previous evidence for the existence of a large, negative genetic correlation between the two characters. Selection for open-field activity increased the frequency of albinism in the low-activity lines and decreased its frequency in the high-activity lines. The direction of this change is consistent with the known effect of albinism on open-field behavior.

In the latter part of the chapter, the theory of quantitative genetics was summarized briefly. Selection changes the frequency of genes in a population, thereby changing the mean genotypic value. The expected change per generation of selection for polygenic characters is a function of the heritability of the character and the selection differential. When two characters are correlated genetically, selection for one will result in a correlated change in the other. Optimal strategies for generating animal models by selective breeding may be designed; however, formulation of such designs requires at least some familiarity with quantitative genetic theory.

References

Archer, J. The Maudsley reactive and nonreactive strains of rats: The need for an objective evaluation of differences. *Behav Genet*, 5:411-413, 1975.

Broadhurst, P.L. The Maudsley reactive and nonreactive strains of rats: A clarification. *Behav Genet*, 6:363-365, 1976.

DeFries, J.C. Selective breeding for behavioral and pharmacological response in laboratory mice. In: Gershon, E.S.; Matthyssee, S.; Ciarnello, R.; and Breakefield, X.O., eds. *Genetic strategies in psychobiology and psychiatry*. Pacific Grove, Calif.: Boxwood Press, 1980. pp. 199-214.

DeFries, J.C., and Hegmann, J.P. Genetic analysis of open-field behavior. In: Lindzey, G., and Thiessen, D.D., eds. *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype*. New York: Appleton-Century-Crofts, 1970. pp. 23-56.

DeFries, J.C.; Gervais, M.C.; and Thomas, E.A. Response to 30 generations of selection for open-field activity in laboratory mice. *Behav Genet*, 8:3-13, 1978.

DeFries, J.C.; Hegmann, J.P.; and Weir, M.W. Open-field behavior in mice: Evidence for a major gene effect mediated by the visual system. *Science*, 154:1577-1579, 1966.

DeFries, J.C.; Kuse, A.R.; and Vandenberg, S.G. Genetic correlations, environmental correlations, and behavior. In: Royce, J.R., ed. *Theoretical Advances in Behavior Genetics*. Alphen aan den Rijn, The Netherlands: Sijthoff and Noordhoff, 1979. pp. 389-417.

Falconer, D.S. *Introduction to Quantitative Genetics*. New York: Ronald Press, 1960.

Gaitonde, M.K., and Festing, M.F.W. Brain glutamic acid decarboxylase and open field activity in ten inbred strains of mice. *Brain Res*, 103:617-621, 1976.

George, F.R.; O'Connor, M.F.; DeFries, J.C.; and Collins, A.C. Components of the GABA system in mice selectively bred for differences in open-field behavior. *Brain Res*, 200:85-92, 1980.

Lush, J.L. Intra-sire correlations or regressions of offspring on dam as a method of estimating heritability of characteristics. *Thirty-third Annual Proceedings of the American Society of Animal Production*. 1940. pp. 293-301.

Lush, J.L. Heritability of quantitative characters in farm animals. *Hereditas*, Suppl. Vol., 356-375, 1949.

McClearn, G.E., and DeFries, J.C. *Introduction to Behavioral Genetics*. San Francisco: Freeman, 1973. 349 pp.

Plomin, R.; DeFries, J.C.; and Loehlin, J.C. Genotype-environment interaction and correlation in the analysis of human behavior. *Psychol Bull*, 84:309-322, 1977.

Pollak, E.; Kempthorne, O.; and Bailey, T.B. (eds.). *Proceedings of the International Conference on Quantitative Genetics*. Ames: Iowa State University Press, 1977. 872 pp.

Rick, J.T.; Tunnicliff, G.; Kerkut, G.A.; Fulker, D.W.; Wilcock, J.; and Broadhurst, P.L. GABA production in brain cortex related to activity and avoidance behaviour in eight strains of rat. *Brain Res*, 32:234-238, 1971.

Tunnicliff, G.; Wimer, C.C.; and Wimer, R.E. Brain γ-aminobutyric acid metabolism and behaviour in the mouse and rat. *Gen Pharmacol*, 7:67-69, 1976.

Current Perspectives on Selective Breeding: Theoretical Aspects

R.C. Roberts

Selection may be defined in terms of differential fertility, whereby the individuals in a population contribute unequally to the succeeding generation. However, from a genetic point of view, this definition is quite trivial without two further conditions. First, the breeding individuals must differ from those that do not breed on some genetic criterion, not just for fortuitous reasons of survival or opportunity. Second, the genetic variation that leads to differential fertility must be heritable. By that, we mean the following: It is quite possible to imagine a combination of genes that enhances mating success, but if that gene combination is broken up during gametogenesis, none of the benefit is transmitted to the offspring. If that were the case, then genetically, nothing would happen. Genetic selection implies that the gene content of the offspring generation differs in some respect from that of the parental generation. Because no new genes are created in the process (neglecting for now random mutation), the only effect of selection is to change gene frequencies toward some optimal value. This implies that the relevant, or desirable, genes must be "recognizable," and further that genes already at, or close to, the optimal frequency will have little effect on the selection process. The object of selection is therefore to increase the frequency of genes that occur too infrequently for our liking, and, complementarily, to reduce the frequency of those genes whose position on a chromosome could be occupied by better ones. Thus, if selection is to have any effect, alternative alleles must be available, and this requires genetic variation among individuals. Populations that are genetically homogeneous are not amenable to selection in terms of what we have defined.

366-072 0 - 82 - 4: QL 3

Basic Concepts

Suppose we have a population of mice, in a genetically heterogeneous stock, whose mean body weight at 6 weeks of age is 20 grams. We select for breeding the heaviest individuals, the mean weight of the selected sample being, say, 26 grams. Their offspring are then reared until they reach the same age of 6 weeks, and let us now suppose that these offspring weigh, on average, 22 grams.

This illustrates three technical concepts. First, the superiority of the chosen parents is the selection differential, S, in our case (26 - 20) = 6 grams. Second, the offspring generation is heavier than the mean of the parental generation, in our case by 2 grams. This is the response to selection, R. Third, the ratio of the response to the selection differential, i.e., what we actually obtained over what we tried to obtain, is the heritability, h^2, of the character, which in the above example takes the numerical value of 0.33. Thus

$$\frac{R}{S} = h^2$$

or as it is usually written

$$R = h^2 S, \tag{1}$$

which shows the heritability as the regression of response on selection differential.

This statement calls for two injunctions. First, we determined the heritability of body weight at 6 weeks of age. The character has to be defined precisely; we have said nothing about the heritability of mature weight, or of birthweight, or of weight at 5 or 7 weeks. Second, the heritability is a population parameter, and our estimate refers strictly to the population represented by our sample of mice. It does not apply to somebody else's mice, nor to mousedom at large, far less to rats or elephant seals. We may—indeed, we often do—choose to ignore these injunctions. But in doing so, we should never be unmindful of the possible errors that are thereby introduced.

If we are prepared to assume the approximate normality of the distribution of the measurements of our character, or perhaps find a suitable transformation, we can extend the formulation of the selection differential. This is most easily visualized as truncation selection, though it need not be strictly so, where all animals above a certain level are allowed to breed and those below it are not. The selected tail of the distribution will have its own mean; and from the properties of the normal curve, this mean value can be expressed as so many standard deviation units. Thus

$$S = i\sigma_{\mathrm{p}} \tag{2}$$

where i is the intensity of selection and σ_{p} is the phenotypic standard deviation. The expression for the response can now be written

$$R = ih^2 \sigma_{\mathrm{p}}, \tag{3}$$

which shows that the magnitude of the response to selection is governed by three factors—the intensity of selection, the heritability of the character, and its phenotypic standard deviation. These will be discussed in turn.

Intensity of Selection

The numerical value of i in the above formulation will increase as the proportion selected becomes smaller. Thus, if only 15 percent of a population are selected, their deviation from the population mean will clearly be greater than if 50 percent were selected, and i correspondingly increases from 0.80 (for 50 percent) to 1.6, thereby doubling the rate of response, other things being equal. These values for i derive directly from tabulations of the normal distribution, though for small samples, discontinuities in the distribution require the use of scores for ordinal (or ranked) data. For example, if only two animals out of four are selected, the first in rank is expected to deviate from the mean by 1.03σ, the second by 0.30σ. The mean deviation of the selected two is therefore 0.67σ, and i takes the value of 0.67. If four are selected out of eight, i becomes 0.72, and the 0.80 quoted above is attained in theory only from an infinitely large population. In practice, however, the departures from the values for infinite populations are negligible once the number of animals measured (not selected) reaches 25 or so.

In practice, the intensity of selection is the main variable under the control of experimenters, and they can select as intensely as they like, subject to the reproductive capacity of the experimental species and considerations of inbreeding (which we defer for the present). Sometimes it may be desirable to wait until more offspring have reached the right age, so that the intensity may be increased. But this may be counterproductive in terms of progress per unit of time. The formula given above shows progress per generation, and a 10 percent gain in i can turn to a loss if the generation interval (measured by equivalent points in the life cycle between generations) is prolonged by more than 10 percent. Thus, in the laboratory mouse, some elementary arithmetic can show that it is often not worthwhile to wait for second or subsequent litters and that experimenters should proceed to the next generation as quickly as possible if they desire to maximize genetic gain per unit time.

Heritability

The term *heritability* seems to have acquired some currency beyond its original technical definition, but that is of no interest to us here. We are concerned with responses to selection in experimental programs, and within that context, heritability is an essential concept, and no formulation is possible without it.

Heritability was presented earlier as the regression of response on selection differential. Though it is not a general feature of regression coefficients, heritability is usually less than unity. There are two reasons why offspring are less good than their selected parents. First, we may express the phenotype (P) as being composed of the genotypic value (G) and an environmental deviation (E) :

$$P = G + E. \tag{4}$$

It is convenient to express E as a deviation, plus or minus, such that its sum over the whole population is zero. This, however, will not be so for a selected sample. Animals chosen as good ones will tend to be good not only for genetic reasons but also because they have a favorable environmental deviation. Because the offspring will reflect only the genetic part, they will tend to deviate less from the population mean than their parents did. The second reason why the offspring will deviate less is that not the whole of G is inherited. The genotypic value reflects the total influence of genes in that individual, such genes' being present in pairs at various loci, and they may further interact with genes at other loci. When the animal breeds, however, its genes are passed on one at a time, and special combinations are broken up. It is only the average effects of the parental genes, considered singly, that the offspring inherit. Thus, meiosis figures as a central concept in the formulation of heritability, so that

$$G = A + NA \tag{5}$$

Where A is the breeding value (alternatively known as the additive effect) and NA is the nonadditive part, representing dominance and interlocular interactions, which are not inherited. The breeding value, A, is measured as the deviation of the offspring from the population mean, i.e., by the response to selection, R. The selection differential, S, is likewise the phenotype, P, of the parents, also considered as a deviation. Therefore, by substituting in formula (1) above,

$$A = h^2 P, \tag{6}$$

which shows the heritability as the regression of breeding value on phenotype (b_{AP}). Thus

$$h^2 = b_{AP} = \frac{cov_{AP}}{V_P} \tag{7}$$

where cov_{AP} is the covariance between A and P and V_P is the phenotypic variance.

To determine the covariance, let us redefine P as

$$P = A + r \tag{8}$$

where r is the remainder, representing all the nonadditive and environmental parts of P, i.e., everything that is not inherited. Then

$$cov_{\mathrm{AP}} = cov_{\mathrm{A(A\,+\,r)}} = V_{\mathrm{A}} + cov_{\mathrm{Ar}} = V_{\mathrm{A}}. \tag{9}$$

It would be a tedious diversion at this point to prove formally that A and r are uncorrelated; a full account can be found in Falconer (1960). Given that, and substituting in formula (7) above

$$h^2 = \frac{V_{\mathrm{A}}}{V_{\mathrm{P}}} \tag{10}$$

which now expresses the heritability as the ratio of two variances—the variance in breeding value (the additive variance) as a proportion of the total phenotypic variance. The ratio, by its nature, varies between 0 and 1, and it indicates how much of the total phenotypic variance is composed of variance in breeding value, i.e., how much of the observed variation in a population is heritable.

The Phenotypic Standard Deviation

Phenotypic standard deviation is the third factor that influences the response to selection. We have just seen how it also enters into the second term of formula (3), the heritability. Ignoring this for the moment, we may note that, other things being equal, the greater the phenotypic variance, the greater the response. However, the qualification "other things being equal" is a hypothetical one, for they never are. Leaving i aside, because it is independent of the other two, it is more profitable to consider the product $h^2\sigma_{\mathrm{P}}$ than it is to take the terms singly. By substituting for h^2 from formula (10), this product becomes $V_{\mathrm{A}}/\sigma_{\mathrm{P}}$, which rather reverses the outlook. For a given V_{A}, we see that the response varies *inversely* with σ_{P}, and the reason may be appreciated from the following. In the same way as we regarded P as consisting of several parts,

$$P = A + NA + E, \tag{11}$$

so we may regard the corresponding variances,

$$V_P = V_A + V_{NA} + V_E \quad , (12)$$

the covariance terms, as noted earlier, being zero. To maximize the response, we therefore need to reduce V_P to its minimum while leaving V_A alone. We cannot do anything about the nonadditive variance, but it may be possible to reduce V_E, the environmental variance by standardizing the environment, reducing measurement error, minimizing disease, etc. In other words, we cut out all the white noise we can, and the response to selection will in consequence be greater. Quite often, though, despite our best endeavors, we are left with a large environmental component of variance, which by combining formulas (10) and (12) leads to a low heritability. While this does not augur well for a selection program, all is not necessarily lost. If we revert to our product in its original form $(h^2\sigma_P)$, a low heritability can still lead to progress if it is associated with a high phenotypic variance. There are traits, e.g., various measures of fertility in domestic livestock, with very low heritabilities but which are nevertheless amenable to genetic improvement because individuals vary so enormously among themselves; i.e., we have a high phenotypic standard deviation.

We should not, in any event, overestimate the benefits of reducing environmental variance. Consider a trait whose phenotypic components are as follows: $V_A = 3, V_{NA} = 3, V_E = 4$ (approximate values for body weight in the mouse, in grams squared). The total phenotypic variance is therefore 10, and the heritability is 0.3. Because

$$R = i\,h^2\,\sigma_P = \frac{i\,V_A}{\sigma_P} \quad (13)$$

as shown earlier, we can calculate relative responses as V_E is reduced, with the consequent reduction in σ_P, as follows:

$$V_E = 4\sigma_P = 3.16R_1 = 0.95i$$

$$V_E = 2\sigma_P = 2.83R_2 = 1.06i = R_1 \times 1.12$$

$$V_E = 0\sigma_P = 2.45R_3 = 1.22i = R_1 \times 1.29.$$

In practical terms, we could, perhaps, at enormous cost and with difficulty, make it feasible to reduce the environmental variance to about half its present value, merely to increase the response by 12 percent. As we shall discuss later, there are probably better and less expensive ways of achieving this modest objective.

Measuring Responses to Selection

Earlier we discussed the main features of selection as a one-step process. Parents were selected on the basis of their phenotype in one generation, and the phenotype of their progeny was measured in the next. In practice, this approach needs some refinement. Single-generation results tend to be erratic, especially with small laboratory populations. The accuracy is increased by repeating the process over several generations, cumulating the response and the selection differential as we go. If we plot these cumulated points, generation by generation, we can obtain a much better idea of progress. Further, the least-squares regression of response on selection differential gives a much more precise estimate of the realized heritability than an estimate from a single generation. The realized heritability—what we actually observe in practice—constitutes the best prediction of what would happen if the process were to be repeated.

There are two more difficulties in measuring the response. The first is to obtain an accurate estimate of the baseline from which we start. The second is to monitor any change in the environment as we proceed. Some major changes in the diet or in the incidence of a disease may be obvious, though perhaps difficult to quantify. Other changes may be more subtle, possibly fluctuating or seasonal, or perhaps appearing as a gradual trend over time, which is unlikely to be detected in the short term. Genetic changes cannot be measured without removing the effects of environmental influences. The method of coping with both the base level and changes in the environment is through the establishment of an unselected control line, where matings are purely random. This gives a series of points corresponding to the base level, and the selected line is measured in terms of its deviation from the contemporaneous control, to remove at least the grosser environmental changes. The regression of the control level on generation number can be used to test any time trend in the environment.

The establishment of a control line, however, is only a partial answer to the problems. The means of the selected line and the control line are both subject to sampling errors, and as Hill (1971) showed, such errors are cumulative. The lines may therefore diverge purely because of accidents of sampling, and the cumulative effect over time in a finite population can be substantial. Any pair of lines, strictly speaking, should be regarded as only one possible outcome, and the selection program should be replicated if facile a view of this solution. If the facilities for conducting a selection experiment are subject to physical limits, there is little point in conducting two such experiments each half as big. The smaller the line, the more subject the line becomes to drift variance, as it is called, and the only argument for replicating within fixed resources is that the magnitude of the drift variance may be estimated. (The principles of replication are discussed much more fully by Falconer 1973, who describes a replication selection experiment for body weight in the mouse.)

There is one other tactic we may use to facilitate the measurement of the response. This is divergent selection, whereby we select for both high and low manifestation of the trait simultaneously. This, in principle, doubles the expected response, and on general grounds, it is easier to measure a big effect than a small one. The difficulty here is that the responses in the two directions may not be symmetrical, as will be discussed later, though this phenomenon itself may be a valid subject of inquiry.

Though this section is about the measurement of the response, we may note here a refinement of the measurement of the selection differential, against which the response is assessed. The process of selection was viewed earlier as differential fertility, and in laboratory studies, experimenters decide which animals are to be allowed to breed. But among the selected sample, there may be further differences in fertility beyond the experimenters' control. Any sterile animals may as well not have been selected at all and, retrospectively, should be excluded from the calculation of the selection differential. Further, of the animals that do breed, the number of offspring that they contribute to the next generation will vary. In calculating the selection differential, the deviation of each selected individual should be weighted by the number of offspring that it provides for measurement in the next generation.

The selection differential may not be the same in the two parental sexes, either by chance or because the variances differ, or indeed, from deliberate policy; e.g., it is often possible to select more intensely among males and then mate each male to several females. However, by the nature of the process of fertilization, the two sexes contribute equal numbers of genes to the next generation. The selection differential should therefore be calculated separately for the two sexes, as weighted deviations from their own sex mean. The overall selection differential is the unweighted arithmetic mean of the two sexes.

Methods of Selection

Individual Selection

Individual selection is the familiar case where individuals are chosen for breeding on their own individual merit, on the assumption that their own phenotype is at least an adequate indication of their breeding value—a statement that will make more sense when the alternatives are discussed below. It is probably the simplest method to operate in practice, and conceptually, it is easier to develop selection theory in its context, as we have been doing. The selected individuals are usually mated at random among themselves, sometimes (as with, say, *Drosophila*) without assignment to specific pairs or mating groups. Individual selection is alternatively known as mass selection; there is no generally accepted distinction between the two terms.

Between-Family Selection

Between-family selection is often abbreviated to family selection, where whole families are chosen to provide breeding individuals, at the expense of other families which are rejected and which do not contribute to further generations. Thus, individuals are chosen to breed because they belong to a good family; their own performance may be good, bad, or indifferent, and is ignored. The rationale for this procedure is that where the heritability is low, an individual's phenotype is a poor guide to its breeding value. In such cases, the family mean is a more reliable guide to the expected breeding value than the individual's own phenotype, which will be largely determined by an environmental deviation of unknown amount and direction. The assumption must be that, over all members of a family, such deviations tend to cancel out. Because of this, the means of large families are more accurately determined than those of small ones.

Family selection thus introduces a new concept—that we take into account information on relatives in assessing an individual's genetic merit. In practice, though, family selection is of little importance, especially in the case of laboratory mammals, for two related reasons. First, the method is very demanding in terms of space, because the majority of families must be discarded to achieve any reasonable selection differential. Second, because the stock is propagated from rather few families, some inbreeding becomes unavoidable, with undesirable consequences, particularly for fertility which will itself aggravate the problem. Family selection, as a method, must be regarded very much as a last resort, where the heritability is too low to provide a desired genetic change by any other means. In which case, the experimenter might ponder whether it is worthwhile to attempt to exploit such meager genetic variation.

There are, however, two modifications of the principle of family selection that may find an application. These may occur where some trait can be measured in one sex only, usually some aspect of reproductive or maternal performance. Alternatively, an animal may have to be killed before some measurement can be taken. In this case, sib selection, i.e., animals are selected on the performance of their sibs, may circumvent the difficulties. Another method, more commonly used in livestock breeding, is a progeny test, a good example being the selection of dairy bulls based on the level of milk production of their daughters. While this method in effect estimates the breeding value directly, its efficiency is greatly eroded by inordinately prolonging the generation interval. In both sib selection and progeny testing, animals are selected for breeding on the basis of performance in specified relatives. Indeed, any relative will provide some information on an animal's expected breeding value. It will be appreciated intuitively that such information must be weighted appropriately (the reader is referred to Falconer 1960 for further details). Briefly, there are three items that may enter into the calculation (using Falconer's symbols): n, the number in the family; r, the coefficient of genetic relationship (e.g., 1/2 for full sibs or progeny, 1/4 for half sibs, etc.); and *t*, the intraclass correlation.

The intraclass correlation measures the similarity among members of a family and will inevitably include similarities due to genetic reasons and also to environmental influences common to members of a family. Any genetic analysis requires that these two causes of similarity be separated, and experimental design should, whenever possible, take these factors into account. Occasionally they are confounded. Full sibs, for instance, inevitably share the uterine and lactational abilities of their mother, and for many traits, these maternal effects, as they are called, are of substantial magnitude. Other environmental influences may make relatives more alike, notoriously so for many attributes in human families. Common environment will inflate the intraclass correlation, and the differences between families may then be due much more to environmental influences than to any genetic differences.

Within-Family Selection

Obviously there would be no point in selecting between families differing mostly for environmental reasons. The solution in that case is to do the exact opposite—select breeding individuals on the basis of their deviation from their own family mean. They are then being compared with individuals subjected to similar environmental influences, and differences among members of the same family are thus more likely to reflect the genetic differences we seek. But there is a cost, because there is less genetic variation within a family than in the population at large, and the increased accuracy of the method must therefore compensate for this loss of genetic resource. Within-family selection applies only when the intraclass correlation is much greater than would be expected for genetic reasons alone, i.e., where the influence of their common environment is substantial. In fact, on strictly theoretical grounds, the method can seldom be justified, but it does have an additional advantage with laboratory populations that has lent it some popularity. When every family, ideally, contributes the same number of parents for the subsequent generation, inbreeding is reduced, and this is particularly important with laboratory mammals, which perforce are often kept in rather small populations.

The formulation of within-family selection again involves *n*, *r*, and *t* (Falconer 1960). The response to within-family selection (R_w) is related to the response to individual selection (R) by the expression

$$R_w = R(1 - r)\sqrt{\frac{(n-1)}{n(1-t)}} \tag{14}$$

for the same intensity of selection. For purposes of illustration, consider full sibs ($r = 1/2$) and let us postulate an intraclass correlation (t) of 0.75, allowing for considerable similarity due to common environment. The expression then takes the value of

$$R_w = R\sqrt{\frac{n-1}{n}}, \tag{15}$$

which shows that selection within families of full sibs is never better than individual selection unless t exceeds 0.75 and, further, that within-family selection becomes progressively less efficient as n, the number in the family, decreases.

Patterns of Response

Selection usually, though not unconditionally, leads to some response. From the basic formulation

$$R = ih^2\sigma_P$$

it follows that if the response is zero, one of the three factors influencing the response must also be zero, most likely the heritability; the other two factors may be observed directly. Gene frequencies change toward their optimal frequencies, ideally toward fixation (gene frequency = 1) when the population consists entirely of homozygotes for desirable alleles. As the gene frequencies approach fixation, further gains become progressively more difficult to attain until, finally, the response ceases and reaches its limit. These contingencies lead to an asymptotic response curve, and under the idealized condition of complete fixation, there will then be no segregation of genes affecting the character. There will be no genetic variance left in the trait, and its heritability will have been reduced to zero.

In practice, however, there are several reasons why the response may cease long before the hypothetical state of complete fixation has been achieved. There may then be considerable genetic variance remaining for the character, as may occasionally be revealed by reversing the direction of the selection. The following list of contingencies implies no order of importance; the reasons may apply variously to specific situations.

Opposing Natural Selection. A steady state may be achieved when artificial selection is seeking change in one direction, but natural selection counterbalances the selection differential. Extreme phenotypes may fail to breed, or their offspring may fail to survive. Evidence for this would be obtained by relaxing the selection and allowing the animals to breed at random. If natural selection has been opposing the response, the mean of the selected stock would be expected to revert, at least some of the way, toward the unselected level.

Accumulated Inbreeding. Because the population size is often not very large in selected lines, some inbreeding becomes unavoidable, with the consequent increase in homozygosity. The effects of inbreeding may well be augmented by the increasing homozygosity of genes affecting the character, for fixation not only will affect such genes but also will inevitably affect adjoining pieces of chromosome, involving genes not contributing directly to the trait. The effects of inbreeding—of increasing homozygosity—are notoriously deleterious, particularly on various aspects of fertility and viability. Inbreeding may have a direct effect on the character under selection, negating any genetic gain from the selection. Alternatively, or perhaps in addition to this, inbreeding may reduce the selection differential to the point where all available breeders are required to propagate the line, so that no further genetic gain can be expected.

Selection Favoring Heterozygotes. The heterozygote at a locus may exceed in value either homozygote and thus be favored when parents are selected. This contingency is known as overdominance. More commonly, perhaps, a quasi-overdominance may arise if one homozygote is favored in terms of the phenotype but is less fertile or viable than the alternative genotypes at that locus. In that case, the heterozygote may contribute more to overall progress than either homozygote. Selection for bristle scores in *Drosophila* frequently accumulates lethal genes—dominant for bristle score but recessive for the lethality—so that the heterozygotes are increased in frequency. The consequence of selection for overdominance of whatever origin is that gene frequencies settle down at intermediate values, the exact value depending on the relative fitness of the two homozygotes. Note that this will arise only as a direct consequence of selection for heterozygotes. If selection is for intermediate values of the character, on its phenotypic scale of measurement, then the consequences are different. In this latter case, complete fixation is still the expectation, but more or less at random for plus and minus alleles at loci contributing to the character. We may perhaps imagine animals being selected for their tolerance of some noxious substance, but where excessive tolerance demands a change in physiological or biochemical relationships, that change impairs fertility. If that were the case, the selection alleles with a negative effect on the response might be fixed to prevent the response proceeding beyond a certain level.

Genetic Asymmetries. Two genetic aspects of the base population may influence the response to selection. The first is that gene frequencies may not be randomly dispersed among the loci affecting the character; they may, on average, be displaced either toward or away from their optimal frequencies with respect to the phenotypic level we seek. As noted earlier, genes already close to their desired frequencies will yield little response. The second aspect refers to the average dominance of the relevant genes; the dominance may be directed toward or against the desired phenotypic expression. If we are selecting for the dominant alleles, then selection will become progressively less efficient at removing the undesirable recessive alleles as their frequencies are reduced. While the occasional homozygote at a specific locus may have little effect, cumulatively such recessive genes may not be negligible. Falconer (1971) described a selection experiment for litter size in mice, where undesirable recessives were exposed by deliberate inbreeding of the selected line. He calculated that the response to the original selection had been impeded by the presence of perhaps as many as 40 such recessive genes, each of which had an effect of about half a phenotypic standard deviation and was present at a frequency of around 0.2. As a result of the inbreeding, Falconer was able to eliminate an estimated 75 percent of these recessives and thereby increase average litter size from 11.5 to 13. Yet 15 generations of continued selection in the presence of these recessives yielded no further response. Selection favoring recessive genes, on the other hand, is highly efficient, because it can lead to the rapid fixation of the favorable homozygotes.

We have thus noted a number of contingencies, any one of which can slow down the response to selection to the extent of stopping it altogether. Examples of most of them, including the idealized case of complete fixation, have been found in practice (see Roberts 1967*b* for examples). Note also that where we have divergent selection, for high and low expressions of some trait, any of the factors noted above could affect the high and low lines differentially, giving an asymmetrical response. There are additional reasons for possible asymmetry. Selection may generate not only a difference in mean between high and low lines but also a difference in phenotypic variance, with inevitable consequences for the selection differential. Fertility differences may appear between the lines, with similar consequences. And as a final complication, the biological basis of superficially similar measurements may not be the same in the two directions. For instance, Falconer (1963) reported that selection for increased litter size was affected by increasing ovulation rate, whereas the decreased litter size in the low line was due to an increase in embryonic mortality. Where that kind of biological distinction occurs, questions of symmetry do not arise; in any event, there are sufficient reasons for possible asymmetry to render invalid any expectation of symmetry based on simplifying assumptions.

Limits to Selection

The multiplicity of factors outlined in the previous section, any or all of which may affect the limit to selection, clearly precludes any exact quantitative treatment. Any study of selection limits must therefore remain largely empirical (the experimental approaches were illustrated in a series of papers by Roberts 1966*a* , 1966*b* , 1967*a* , 1967*b*). Nevertheless, the theoretical treatment of limits in the idealized case of fixation reveals some basic features of the selection process, as discussed by Robertson (1960) and by Hill and Robertson (1966). A critical concept in the formulation is that of the chance fixation of alleles unfavorable to the direction of selection; that is, a selected line will not necessarily have been fixed for all of the desirable alleles present in the base population. While chance alone may secure this outcome, particularly as population size is reduced, any linkage disequilibrium will aggravate the situation. If two desirable genes are linked in repulsion, then the one with the greater effect on the character will tend to get fixed, at the expense of the other. The implication of chance fixation is that two lines selected in the same direction, even from the same base population, will not be fixed for identical alleles at all loci. Crosses between selected lines that have reached their limits may therefore yield new genetic variance and lead to further responses. (A more detailed discussion of these aspects was provided by Roberts 1974.)

It is inappropriate to provide an extended account here of the theory of selection limits. The main conclusions from Robertson's (1960) paper may be summarized as follows. First, the expected limit is a function only of the product Ni, where N is the effective population size and i is the intensity of selection. The exact form of the function is somewhat complicated, depending on gene frequencies, gene effects, and the type of gene action. At low values of Ni, only genes at a high frequency or of a large effect are reasonably certain to be fixed; as Ni increases, we tend also to fix rarer genes or those of smaller effects. Generally, the larger the population, the greater the total advance, as might be expected. Some idea of the total advance is provided, in principle, from the gain in the first generation. Theoretically, the total gain will equal $2N$ times that in the first generation, for low values of Ni and for genes that act additively; recessive genes will give a greater total response. In practice, this prediction is of limited value because it is difficult to measure the initial gain with sufficient precision.

The next conclusion concerns the duration of the response. Because the response curve is an asymptotic one, this question does not have much meaning if we talk of the total advance. But the "half-life" of the process—the time taken to secure half of the total advance—can be estimated. The half-life will not exceed 1.4 N generations for genes that act additively and 2 N generations for recessive genes. In practice, the half-life can be much less than this (Roberts 1966*a*), under which circumstances most of the desirable alleles will tend to get fixed.

Finally, the greatest total response is achieved when one-half of the population is selected, though the loss is negligible until the proportion is reduced to one-quarter or so, except in small populations. But if the rate of initial response is maximized by selecting very intensely, then this can be achieved only at the expense of the ultimate gain. There is therefore a potential conflict between these two objectives. It is perhaps fortunate that for laboratory mammals their reproductive rate is such that intense selection is not possible in any case, thereby obviating the dilemma.

Falconer (1960) suggested that on empirical evidence the response to selection may last some 20 or 30 generations, yielding a divergence between high and low expressions of the character of perhaps 10 to 20 times the phenotypic standard deviations. While exceptions are plausible in theory and found in practice, there is no real need to revise these estimates as broad expectations.

Correlated Responses

As we select for one character, it is frequently found that other characters change simultaneously. This is known as a correlated response to selection. Thus, for instance, when mice are selected for heavier body weights, they typically ovulate more eggs and possess a much more phlegmatic disposition, among a whole array of other more or less predictable responses. Though we may direct our attention to genes contributing to the character of interest, those selfsame genes will perforce affect other characters, for pleiotropy is universal. This connection between two characters—between two phenotypic measurements—can be quantified in terms of the genetic correlation, r_A, between the two characters. Essentially, this is nothing more than the genetic part of the phenotypic correlation, r_P, between two traits, the exact partitioning being

$$r_P = r_A h_X h_Y + r_E e_X e_Y \tag{16}$$

(for derivation, see Falconer 1960). The symbols had mostly been defined earlier: the subscripts X and Y stand for the two traits, r_E is the environmental correlation, h is the square root of the heritability, and e is similarly derived from its square, $e^2 = (1 - h^2)$. The genetic correlation is the correlation in breeding value for the two traits, and in the absence of a positive environmental association, it will exceed the phenotypic correlation because the term $h_X h_Y$ has an upper numerical value of unity.

Without pursuing algebraic formulations, it is sufficient to note here that if the genes making a mouse grow faster also make it eat more, we could describe this association formally as a genetic correlation between growth rate and food consumption, without gaining any profound insight into what goes on. It may take more imagination to explain why that mouse is also more phlegmatic, but the determination of the genetic correlation would not necessarily help. Genes feed vast biochemical networks, and the outcome at various outlets (phenotypic measurements) are not necessarily intuitively obvious. It is sufficient to note that whenever we begin to modify any one character by selection, we should not be astonished by a concomitant change in another. Particularly if we regard an animal as a product of its evolutionary history, whereby its genetic constitution has been framed in terms of its fitness, then artificial selection—essentially designed to alter its genetic constitution—may well have adverse effects on its fitness. This is why selected lines are so often more difficult to maintain than the unselected stocks from which they are derived. We cannot escape considerations of natural fitness even under the best regulated laboratory conditions. (A further discussion of the biological aspects of correlated responses is given by Roberts 1979, while the theory is expounded by Falconer 1960.)

In principle it is possible to make greater progress in some trait X by selecting not for X itself but for some other trait Y, with which it is correlated. If we may assume equal intensities of selection, it can be shown that this contingency will arise only when $r_A h_Y$ exceeds h_X (symbols as earlier). Simple arithmetic reveals this to be an improbable occurrence unless the genetic correlation is close to unity.

In this section, we have merely noted that correlated responses are a common byproduct of selection procedures. It follows that a line selected for any single trait cannot be automatically regarded as representing the norm for some other trait that might also have been affected by the same selection process.

Selection for More Than One Trait

In the breeding of domestic livestock, a selection program often must take into account several traits simultaneously because each trait contributes to overall economic merit. Among laboratory selection experiments, multiple traits figure much less prominently, partly because the economic considerations do not apply, but mostly because it is in the nature of scientific inquiry to manipulate, where possible, one variable at a time. It is obvious that if the total effect is dissipated over more than one trait, the attention paid to any one of them is reduced in proportion. But in special circumstances, it may be valid to pursue more than one selection objective simultaneously; for example, it may be desirable for certain purposes to increase juvenile weight while reducing mature weight, or to select for the simultaneous resistance to two separate parasites.

There are basically three ways in which this exercise might be attempted. The first is tandem selection, where the traits are subjected to selection successively, either in alternate generations or over longer intervals. The second is independent culling, where a proportion of the breeding stock is selected on its performance with respect to the first trait, and that proportion is then reduced further on the basis of the second trait, etc. The proportions can be modified, if desired, to give more attention to some traits than to others. However, in species whose reproductive rate is low, the scope for selection becomes limited, especially with more than two traits. More efficient than either tandem selection or independent culling is index selection, which combines the phenotypic measurements on all the traits into one figure on some arbitrary scale, that figure then becoming the "phenotype" on which selection decisions are based. The available information must of course be combined appropriately, but the principle of the method is that if an animal is sufficiently superior in one particular respect, it might still be selected even though it is only of moderate interest in other respects. The overall merit is a compound trait whose components are no longer considered singly.

If the component traits are uncorrelated with one another, then the index, I_u, takes the form

$$I_u = h_1^2 P_1 + h_2^2 P_2 + \dots + h_k^2 P_k \tag{17}$$

where P_1 is the phenotypic value for the first trait, etc. The weighting factors are the heritabilities of the individual traits; we need little more than intuition to accept this as reasonable. But if the component traits are not independent, then the weighting factors must be modified accordingly, and the index for correlated traits (I_c) is usually written as

$$I_c = b_1 P_1 + b_2 P_2 + \dots b_k P_k \tag{18}$$

where b_1 is the appropriate weighting factor for the phenotypic value for the first trait, etc. The weighting factors now become somewhat complicated functions of the heritabilities of the component traits and also of both phenotypic and genetic correlations among them. This ignores consideration of any difference in the relative importance of the traits, though this could also be included. We cannot begin to develop the appropriate theory here, nor can we reasonably describe the actual computation of an index—an exercise greatly facilitated by computer routines involving matrix algebra, if the index includes more than two traits. (The reader is referred to Becker

1976 for an excellent account of the computation and also for entry to the literature on the theory.)

If index selection is to prove efficient, it is important that the component traits be weighted appropriately. To achieve this, accurate estimates of the relevant parameters are essential, and full variance-covariance matrices, both phenotypic and genetic, are necessary. From this it can be seen that the application of index selection is contingent upon detailed knowledge of the genetic properties of the component traits, and as such, index selection is purely a manipulative procedure. By the nature of the exercise, it is not designed to provide further genetic knowledge, but merely to apply to maximum advantage what is already known.

The principles of index selection can readily accommodate in the same index information on relatives, in order to assess better the breeding value of an individual. The principles can further be extended to yield what is known as a restricted index, where one or more traits are changed while holding another or others constant. This could be of considerable applied interest, e.g., if an investigator wants to select for tolerance to a drug without simultaneously increasing body weight. At this point, however, the theory becomes difficult and complicated, and its application requires considerable biometrical expertise.

Applications

Much of the theory of selection was developed originally in the context of plant and animal breeding, where the applications are self-evident. In laboratory situations, where an investigator may want to develop specialized lines for specific items of research, the emphasis can be similar. The science of genetics depends critically on the availability of genetic variation, and selection can be employed to exploit such variation by producing divergent lines for the high and low expression of some trait. The potential power of such material in biological analysis needs no emphasis. A well-known example from the behavioral sciences is the widespread employment of Tryon's maze-bright and maze-dull rats, while lines of mice selected for growth rate or for fertility have been used for innumerable studies on those traits.

Selection can also be employed as a means of genetic analysis in its own right, and it is a vital step in the description of the genetic architecture of various traits. Because, in the past, natural selection will have operated on traits closely related to fitness, any additive variance in such traits will have been largely depleted. By the same token, interactions and nonadditive genetic variance due to dominance are prominent features of those traits. For this reason, traits close to fitness, such as fertility and viability, have low heritabilities and respond poorly to selection but are very susceptible to inbreeding, thus reflecting their nonadditive variance. Selection, among the other methodologies of quantitative genetics, can thus provide insight into the consequences of natural selection and evolution, and the relevance of these considerations is beyond dispute.

References

Becker, W.A. *Manual of Procedures in Quantitative Genetics.* 3rd ed. Pullman: Washington State University Press, 1976.

Falconer, D.S. *Introduction to Quantitative Genetics.* Edinburgh: Oliver and Boyd, 1960.

Falconer, D.S. Qualitatively different responses to selection in opposite directions. In: Hanson, W.D., and Robinson, H.F., eds. *Statistical Genetics and Plant Breeding* (No. 982). Washington: National Research Council of the National Academy of Sciences, 1963. pp. 487-490.

Falconer, D.S. Improvement of litter size in a strain of mice at a selection limit. *Genet Res,* 17:215-235, 1971.

Falconer, D.S. Replicated selection for body weight in mice. *Genet Res,* 22:291-321, 1973.

Hill, W.G. Design and efficiency of selection experiments for estimating genetic parameters. *Biometrics,* 27:293-311, 1971.

Hill, W.G., and Robertson, A. The effect of linkage on limits to artificial selection. *Genet Res,* 8:269-294, 1966.

Roberts, R.C. The limits to artificial selection for body weight in the mouse. I. The limits attained in earlier experiments. *Genet Res,* 8:347-360, 1966*a.*

Roberts, R.C. The limits to artificial selection for body weight in the mouse. II. The genetic nature of the limits. *Genet Res,* 8:361-375, 1966*b.*

Roberts, R.C. The limits to artificial selection for body weight in the mouse. III. Selection from crosses between previously selected lines. *Genet Res,* 9:73-85, 1967*a.*

Roberts, R.C. The limits to artificial selection for body weight in the mouse. IV. Sources of new genetic variance–Irradiation and outcrossing. *Genet Res,* 9:87-98, 1967*b.*

Roberts, R.C. Selection limits in the mouse and their relevance to animal breeding. *Proceedings of lst World Congress on Genetics Applied to Livestock Production.* Madrid: 1974.

Roberts, R.C. Side effects of selection for growth in laboratory animals. *Livestock Prod Sci,* 6:93-104, 1979.

Robertson, A. A theory of limits in artificial selection. *Proc R Soc, B,* 153:234-249, 1960.

A Review of Selective Breeding Programs*

Janet Shibley Hyde

The purpose of this chapter is to review the behavioral selective breeding programs that have been done and to provide an idea of the kinds of behaviors that have been investigated, the techniques that have been used, and the kinds of results that can be obtained. First, some of the early, historic selective breeding programs will be described (McClearn 1962). This chapter will abandon the historic approach because many researchers in the 1960s and 1970s were found to be using designs that were no more sophisticated than the ones that had been used in the 1920s and 1930s. The first part of the chapter will concentrate on studies with mice and rats; the last part will focus on studies with *Drosophila*. Discussions of alcohol-related studies are omitted here because they are covered in detail in other chapters.

Early Studies

Maze Brightness and Maze Dullness

The eminent psychologist Edward C. Tolman was one of the first to recognize the importance of using selective breeding and other genetic methods in the scientific analysis of behavior. He commented:

> The problem of this investigation might appear to be a matter of concern primarily for the geneticist. Nonetheless, it is also one of very great interest to the psychologist. For could we, as geneticists, discover the complete genetic mecha-

---

*Preparation of this chapter was supported in part by grant MH-28374 from the National Institutes of Health.

> nism of a character such as maze-learning ability—i.e., how many genes it involves, how these segregate, what their linkages are, etc.—we would necessarily, at the same time, be discovering what psychologically, or behavioristically, maze-learning ability may be said to be made up of, what component abilities it contains, whether these vary independently of one another, what their relations are to other measurable abilities, as say, sensory discrimination, nervousness, etc. The answers to the genetic problem require the answers to the psychological, while at the same time, the answers to the former point the way to those of the latter. (1924, p. 1)

Tolman certainly had a marvelous vision of the direction of behavior-genetic research. It is disquieting to realize how little progress we have made toward those goals in the 50 years since he outlined them. On the other hand, particularly in some of the studies with *Drosophila* to be discussed later, remarkably sophisticated analyses have been done that have gone a long way toward reaching the goals outlined by Tolman.

As his own contribution, Tolman (1924) began a program of selection for maze brightness and maze dullness in rats. He started with a heterogeneous group of animals and tested them in a maze. On the basis of their performance (running time, errors, and perfect runs), Tolman then selected animals to form a bright line and a dull line. The study was continued for only two generations. The results in the first generation were quite exciting—the offspring of the brights performed considerably better in the maze than did the offspring of the dulls. For example, the mean number of errors in the maze was 15.7 for the offspring of the dulls but only 6.2 for the offspring of the brights. In the second generation, though, the lines converged somewhat. The study was apparently terminated at that point. Two problems may have contributed to the deterioration in the results: (1) the maze that was used for measurement had flaws that made it unreliable and (2) there was probably some brother-sister mating, which created inbreeding depression. It is interesting to note, though, that the necessity of bidirectional selection was realized even in this early study.

Tryon (1942), a student and later a colleague of Tolman's at Berkeley, took the idea of selection for maze-learning ability and built it into one of the classic studies of behavior genetics. Tryon published data for eight generations and continued selection even past that. Like Tolman, he used bidirectional selection. He made a major improvement, however, by designing an automated, lengthy maze that made measurement considerably more reliable. He quickly got good separation between the lines and the differences held up in later generations. Indeed, by the eighth generation of selection there was virtually no overlap in the distributions of error scores for the bright line and the dull line.

At about the same time, Heron (1935, 1941) was also selecting for maze-learning ability in rats, using a different automated maze. He, too, was successful in achieving good separation between the two lines. Eventually he reported results through 16 generations of selection.

Those outside the field of psychology may wonder why the early behavior geneticists were so fixated on the study of maze-learning ability. The work apparently sprang from an interest in genetic influences on human intelligence, a topic that was in the throes of the nature-nurture debate. The strategy then was to use animal models for research, and maze-learning ability was essentially thought to represent rat "intelligence."

There was some question, though, as to whether the mazes that had been used really did measure rat intelligence (some of the disconcerting findings will be discussed later). One other selection program attempted to get at something that could more properly be regarded as rat intelligence.

Rat Intelligence

Thompson (1954) attempted to measure what might more validly be called rat intelligence by using the Hebb-Williams maze, which presents the animals with 24 different problems that sample a broad range of learning abilities. Thus, it was thought to be more analogous to human intelligence tests. Because Thompson believed that it was desirable for the lines to be as homozygous as possible, inbreeding was practiced.

After two generations of selection the two lines diverged sharply, and good results were obtained in the six generations of selection that were reported. Thompson also noted that there were marked problems with fertility in the brother-sister matings, indicating that the practice of inbreeding has some serious disadvantages.

Not all of the early selection studies focused on rat intelligence. Two other studies attempted to investigate animal analogs of human "personality" or "temperament."

Activity

Rundquist (1933) selected for motor activity in rats as measured by number of revolutions from running in an activity wheel. For the first five generations, Rundquist pooled all offspring and selected highs and lows. After the fifth generation, the two lines were separated; highs were chosen from the high-line offspring, lows from the low. Inbreeding was practiced sporadically. Marked differences between the actives and inactives were apparent even in the first and second generations and continued throughout. It was apparently somewhat easier to select for low activity than for high activity. This was more true for males than for females, however. The disparate results for females might be attributed to estrous cycle fluctuations in activity level, which have been well documented. Estrous cycle variations may be regarded as a phenomenon of interest in their own right. In a selection study, however, they become a source of unreliability of measurement, a point that will be raised again in this chapter in a discussion of research on aggression in female mice.

Emotionality

Hall (1938, 1951) selected for emotionality in rats. His operational definition of "emotionality" was urination and defecation on 12 daily 2-minute trials in a brightly lit open field. The high-emotionality (high scores) and low-emotionality lines were separated and breeding was done within lines from the beginning, with selection continuing for 12 generations. Increases in emotionality in the high line occurred until about the ninth generation, after which scores leveled off. Scores in the low line were low after the first generation of selection but never dropped below that point, possibly because of a kind of "floor effect" in measurement; that is, from the first generation this line usually showed urination or defecation on only 1 of the 12 days of testing. For those who accepted Hall's measure as valid, this study provided a classic demonstration of the inheritance of emotionality.

Negative Results

The discussion to this point, and most of the succeeding discussion, might give one the impression that all behavioral selective breeding programs succeed, i.e., that divergent lines are successfully established. From this one might conclude that all behaviors (or at least all those that have been tried) are under at least some genetic control. These observations are fairly valid. Nevertheless, two early negative results from selection attempts and one more recent one should be noted (Fuller and Thompson 1960).

McDougall was unable to increase the learning ability of rats through selective breeding (Rhine and McDougall 1933). However, because the investigator's purpose in conducting the study was to "prove" the Lamarckian hypothesis, it seems possible that some experimenter bias may have affected the results.

In another instance, 10 generations of selective breeding for performance in a simple water maze were not successful in producing behavioral changes (Kuppaswany 1947).

Ginsburg (1967) reported on three later selection studies that failed. The selected behaviors were aggression, emotionality, and susceptibility to audiogenic seizures. However, in every case the foundation population was the C57BL/10 inbred strain of mice. Because there should be essentially no genetic variability in an inbred strain, it is not surprising that it would be impossible to conduct a successful selective breeding program.

The Midlife of Selection Studies

After the initial group of early selection studies, there followed a group of studies, in the 1950s and early 1960s, marked not by increases in sophistication of genetic methods—simple bidirectional selection was still the rule—but rather by a diversification to other important and interesting behaviors.

Reactivity/Nonreactivity

Beginning in 1954, Broadhurst practiced bidirectional selection for emotional elimination in a restandardized version of Hall's open-field test. The result was the Maudsley reactive (MR) and Maudsley nonreactive (MNR) strains of rats (for reviews, see Broadhurst 1975, 1960). Based on extensive auxiliary experiments, Broadhurst argued that the two lines represent two extremes of the emotionality phenotype. Separation between the lines was substantial after only two generations of selection.

Audiogenic Seizure Susceptibility

Audiogenic seizures are among the behaviors that have received thorough behavior-genetic analyses. Audiogenic seizures are seizures induced by high-intensity sounds. The seizure itself may consist of frenzied running, clonic convulsions, tonic convulsions, and death. Various investigators have provided evidence for single major-gene effects on the behavior and for polygenic inheritance. Physiological mechanisms have also been investigated extensively. Among the genetic analyses was an early selection program by Frings and Frings (1953; see also Griffiths 1942; Maier 1943). They began with a heterogeneous stock of albino mice (although one wonders here, as in some other studies, how heterogeneous a stock of all albino mice really is). After only two generations of selection, the percentage of susceptibles had increased greatly, almost to the maximum attained. However, the latency to seizure continued to decrease for seven generations, and the age of susceptibility also fell. These results emphasize the importance of a multivariate approach, with multiple behavioral measures, in a selection program. The dichotomous seizure/no seizure variable, seizure latencies, and age of susceptibility all showed somewhat different patterns of response to selection, although all responded. The last variable also highlights the importance of considering and controlling developmental variables. A given animal may be highly susceptible to seizures at one age and not at all susceptible at another age. A sophisticated study by Deckard et al. (1976), to be discussed later, investigated the developmental and environmental aspects of this behavior.

Male Aggression

Finnish psychologist Kirsti Lagerspetz and her colleagues have selected for male aggressiveness in mice (Lagerspetz 1964; Lagerspetz and Lagerspetz 1971). The foundation population was a heterogeneous stock of albino mice, although here again one questions how much genetic variability is present in such a population. Selection was bidirectional and a response to selection was obtained in both lines, with the high line increasing in aggressiveness and the low line decreasing in aggressiveness. The criterion for selection was a rating of aggressiveness on a 7-point scale when the mouse was placed in a paired encounter situation. Note that the selection was single sex—only males were tested—apparently because fighting behavior could not be obtained in females. Inbreeding was practiced systematically in the early generations in the hope that it would produce a faster response to selection. Although data have been reported for 19 generations of selection, the limits of selection appear to have been reached by the seventh selected generation. The limits may have been reached so early because the inbreeding eliminated genetic variability and thus eliminated the potential for later gains from selection.

Rearing Behavior

Van Abeelen and his colleagues have selected for frequency of exploratory rearing behavior (animal rears up on hind legs) in mice (van Abeelen 1970; van Abeelen et al. 1973). The foundation population was the F_2 of a cross between inbred strains C57BL/6 and DBA/2. A high-scoring line and a low-scoring line were produced "by using a system of repeated backcrossing and inbreeding with concomitant directional selection" (1973, p. 85). Selection through 13 generations has been reported. Animals in both lines became homozygous for the dilute allele and the brown allele; in the low line, this occurred by the second generation; in the high line the dilute locus was fixed by S_4 and the brown allele was fixed by S_{11}. Because these loci are fixed, variation in rearing behavior between the two lines must be attributed to other loci.

Saccharin Preference

Nachman (1959) selectively bred rats, the criterion for selection being their preference for a saccharin solution (saccharin-bred line) or water (water-bred line). Results were reported for two generations, and the lines were already quite divergent. In the second generation, differences between the lines were found in saccharin solution versus water preference at intermediate concentrations, but not at extremely strong (2.0 percent) or extremely weak (0.05 percent and 0.10 percent) concentrations.

T-Maze Performance

Hurnik et al. (1973) selected for high and low speed of running a two-unit T-maze for social motivation (sibling of the subject was in the goal box). Results for five generations of selection were reported, beginning with a foundation population of the F_2 of the inbred strains SWR and C57BL. Selection was based on an index of both individual and family performance. Although the heritability of the trait based on intraclass correlation of full sibs was low (0.13), a satisfactory response to selection was obtained nonetheless. The limits of selection were reached more quickly in the line selected for slower running. Differences between the lines were significant for number of errorless trials.

Other studies involving selective breeding for learning include those by Bignami (1965) and Bovet et al. (1969).

Recent Studies with Sophisticated Genetic Designs

Following the middle group of selection studies, recent studies have successfully used more sophisticated genetic designs that provide more data and more powerful tests of hypotheses.

Activity

DeFries and his colleagues have successfully selected for open-field activity in mice. An early report details results for the first 10 generations of selection (DeFries and Hegmann 1970; DeFries et al. 1970), while a recent report provides data for a total of 30 generations of selection (DeFries et al. 1978). Several features of this study make it the model for the modern, sophisticated selection design: (1) An unselected control line, in addition to high and low lines, is used (note that the line is unselected, not selected for intermediate values). (2) A replicate of all three lines is also formed, for a total of six lines; the replication is invaluable for helping one to know how firm conclusions should be and for assessing the presence or absence of correlated responses to selection. (3) Selection is within families, with no sib matings. This minimizes inbreeding and thereby maintains genetic variability and the potential for continued response to selection. With the relatively small populations used in selection studies there must be some rate of inbreeding, but this design keeps it very low. (4) The study has now proceeded for 30 generations, making it the longest behavioral selection study on a mammal.

The total number of interruptions of photocell beams in an open field on two 3-minute tests on 2 consecutive days was the basis of selection. Number of fecal boluses was also recorded. In the 30 generations of testing, 14,184 animals contributed data.

Separation between the lines was fairly good beginning at about S_7, and the response to selection has continued through 30 generations. This appears to be a tribute to the success of a design that minimizes inbreeding. In generation 30, the activity of the high lines was about 30 times that of the low lines. The realized heritability for the first 10 generations was 0.13. It is worth noting that response to selection has been excellent despite this low heritability.

Female Aggression

Several recent studies have used the DeFries six-line design. Among these is the program of selection for female aggression in mice by Hyde and her colleagues (Ebert and Hyde 1976; Hyde and Ebert 1976; Hyde and Sawyer 1980). Unlike the DeFries study, in which the foundation population was the F_3 of inbred strains BALB/cJ and C57BL/6J, Hyde began with a foundation population of wild-trapped animals more closely approximating the kinds of populations on which natural selection acts. Further, the behavior in question, interfemale isolation-induced fighting, is at best rare in laboratory animals, but fairly common among wild house mice. The criterion for selection was the rating on a 5-point scale, modified from that used by Lagerspetz (1964), following a 7-minute encounter with another female mouse. Note that selection is single sex.

Good separation between the lines has been obtained. About 25 percent of the foundation population attacked. As of the S_{12} generation, the percentage of attacks was zero or near-zero in the low lines and 50 percent or close to it in the high lines. Realized heritabilities for the first eight generations of selection were: H1, 0.12; L1, 0.34; H2, 0.14; and L2, 0.46.

In an auxiliary experiment, significant estrous cycle variations in female aggressive behavior were discovered (Hyde and Sawyer 1977). This may be a source of uncontrolled unreliability in the behavioral measurements in this selection program.

Learning

Two recent selection programs for learning ability in mice have been reported. Smith (1978) selected for shuttle avoidance for five generations using a modification of the DeFries design as extended by Hyde, with five lines (only one control line) beginning from a wild population. Separation of lines was good in the fifth generation, although before that there was some crossing over of the lines. The heritability for the high lines was 0.12; for the low lines, 0.16.

Festing (1973*b*) also used the six-line design and within-family selection for speed of water escape learning. Results for four generations of selection were presented, and separation among lines was not good until the fourth generation. Realized heritability was 0.065. This particular study is interesting because its results can be compared with results of other genetic analyses of the same behavior by the same investigator (Festing 1973*a*, 1974).

Acoustic Priming

In an extension of earlier work on audiogenic seizure susceptibility, Deckard et al. (1976; see also Chen and Fuller 1975) selectively bred for acoustic priming in mice. "Acoustic priming" refers to the phenomenon of mice from seizure-resistant strains being made seizure susceptible by exposure to intense sounds. Animals were primed at 19 days of age and tested at 22 and 27 days of age. Selection was on the basis of a rating from 0 (no response) to 4 (lethal seizure). High, low, and unselected control lines were formed. Results for eight generations of selection were reported, and separation of the lines was good.

This study is particularly elegant in its ability to explore genetic determinants of developmental processes and of susceptibility to environmental influences. Further, the study helps to resolve some of the conflicting literature on audiogenic seizures. Several investigators have found single-gene effects on the behavior, while others have found the effects to be polygenic. Deckard et al. (1976) tested to see whether first-trial seizures with no priming had shown a correlated response to selection; they had not. This suggests that first-trial and priming-induced seizures are affected by different genes.

Behavioral Correlates of Selection for Physical Characters

To this point the discussion has involved selective breeding in which the criterion for selection was a behavioral measure. Once the selected lines are established, one can begin to investigate the physiological control of the behavior (as will be discussed in a later section of this chapter). However, the reverse approach is also worth noting—namely, selecting for some physical character that is thought to have behavioral significance and then looking at behavioral correlated responses to selection. The following are some examples of this approach.

Blood Pressure

Elias and coworkers (Elias and Schlager 1974; Elias et al. 1975) studied aggressiveness and discrimination learning in mice selectively bred for high and low blood pressure.

Thyroid Function

Blizard and Chai (1972) studied the behavioral correlates of selection for thyroid function in mice. They found that the LIR line (relatively hypothyroid) defecated significantly more and had shorter latencies to move in an open field; they also learned a water maze faster than did the HIR (hyperthyroid) strain. The results of this study were consistent with those obtained with the reverse approach—the Maudsley reactive or high-defecation strain was hypothyroid relative to the nonreactive strain. That is, hypothyroidism seems to be associated with high emotionality or, at least, defecation.

Brain Weight

The Roderick-Wimer brain weight lines were selected on the basis of absolute brain weight. Replicated high, low, and control lines were formed. Unfortunately, the fixation of retinal degeneration in the low lines and one of the high lines in generation 6 complicated behavioral testing. Nonetheless, high brain weight mice have been shown to be superior at discrimination learning (Wimer and Prater

1966), at spatial discrimination reversal learning (Elias 1969), and on brightness discrimination and active avoidance tasks (Wimer et al. 1969). Their performance on a passive avoidance task was inferior (Wimer et al. 1969).

Collins (1970) found that mice selected for high brain weight were significantly less aggressive than those selected for low brain weight. The activity level of high brain weight animals was also higher (Wimer et al. 1969).

Further Analyses of Selected Lines

Once selected lines have been established, a number of different kinds of followup analyses can, and should, be done.

Checking On What Has Been Selected

Most behavioral measures tap complex domains. An investigator usually claims to have selected for a particular construct based on a single behavioral measure. It is wise to verify that what really differentiates the lines is that construct rather than some spurious differences.

The classic example of this approach is in the followup studies of Tryon's selection for maze brightness and maze dullness in rats. Although Tryon specifically stated that he had not intended to select for "general intelligence," it has often been assumed that that was his intention. As described previously, he used performance in a single maze to measure maze brightness and maze dullness. When Searle (1949) tested the Tryon lines on some other measures of learning, he found that the dulls did better than the brights on some tasks. It therefore appears that Tryon indeed had not selected for rat "general intelligence," but rather for a more specific ability in a specific maze.

Genetic Analysis of Selected Lines

Genetic analyses of the selected lines may be done to determine whether they are homozygous or whether behavioral differences are due to a single locus or are polygenic. An an example, Tryon (1940) crossed his bright and dull lines to form an F_1 and F_2. The F_2 showed performance intermediate between the bright and dull lines. Because the F_1 was about as variable as the F_2, it seems likely that the bright and dull lines were not homozygous and that brightness and dullness were polygenically controlled.

Another example is the diallel analysis of the six lines of the DeFries activity selection program (Halcomb et al. 1975; Hewitt et al. 1977). Results indicated that the genes responsible for the line differences in open-field activity had primarily additive effects.

Hypothesis Testing

The artificial selection method is usually not well designed for hypothesis testing, but rather provides correlational data. In some cases, however, selection programs may provide hypothesis-testing or hypothesis-confirming data for well-defined genetic hypotheses.

For example, evidence from multiple sources indicates that the albino allele in mice (and some other species) has a major-gene effect on activity, with activity being reduced by the albino allele. In the DeFries selection program for activity (DeFries et al. 1970), one of the low lines became fixed for the albino gene in the eighth generation, and the other soon afterward. The percentage of albinos was intermediate in the control lines and low in the high-activity lines. These data certainly support the hypothesis of a major-gene effect of the albino gene on activity level.

Physiological Mechanisms

It is presumed that selective breeding produces genotypic differences between lines that, in turn, produce behavioral differences. In analyzing the gene-behavior relationship, it is of considerable importance to investigate the physiological intermediaries between the genes and the behavior. On the simplest level, this can be done by looking at physiological differences between the selected lines. The following are some examples of that approach.

The Tryon maze-bright line was found to have a higher level of cholinesterase activity in the cerebral cortex than the maze-dull line (Krech et al. 1956). However, it was later shown that this was a fortuitous association (Rosenzweig et al. 1958). The problem of fortuitous physiological differences between selected lines must be kept in mind when investigating physiological mechanisms. A number of reasons may exist for such fortuitous relationships; in particular, if much inbreeding occurs, some alleles may accidentally become fixed even though they have nothing to do with the behavior in question. Replicated selected lines such as those in the DeFries design help to guard against this problem.

In lines selected for high and low levels of male aggressiveness, Lagerspetz et al. (1968) reported that males of the aggressive line had lower serotonin levels in the forebrain, higher noradrenaline levels in the brain stem, and heavier testes than males from the unaggressive line.

Drosophila Studies

The other major species in which behavioral selective breeding has been done is *Drosophila*. (There have been behavioral selection studies of a few other miscellaneous species, including aggressiveness in chickens—Guhl et al. 1960, social dominance in chickens—Craig, et al. 1965, mating ability in chickens—Siegel 1965, responses to imprinting in chicks (Graves and Siegel 1969), and mating behavior in Japanese quail—Cunningham and Siegel 1978.) *Drosophila* offers a number of advantages over other species in behavior-genetic analyses. The intergeneration interval is short, so that an extensive selective breeding program can be done in a matter of weeks. The animals are small and cheaply maintained, and they can be mass tested; thus, it is quite feasible to test thousands of subjects. Their genetic architecture has been studied extensively; on the other hand, their behavioral repertoire is rather limited. They do move about and mate, however, and these behaviors have been well studied. Another disadvantage is that, because they are not mammals, it is more difficult to generalize results from *Drosophila* to humans.

Geotaxis

Geotaxis is gravity-oriented locomotion—that is, movement with gravity or against gravity. It is the behavioral trait that has received the most complete genetic analysis to date (Dobzhansky and Spassky 1962; Ehrman and Parsons 1976; Erlenmeyer-Kimling et al. 1962; Hirsch 1963; Watanabe 1976). Geotaxis is measured by placing animals in a 10- or 15-unit maze fixed vertically; mass testing can be done efficiently. Selection for positive and negative geotaxis has been successful (Hirsch 1963).

Erlenmeyer-Kimling et al. (1962) were able to analyze the role of the three major chromosomes possessed by *D. melanogaster* in the response to selection for geotaxis. This was done by crossing the selected lines with tester stocks carrying dominant genes with inversions that inhibit crossing over in that chromosome. This can be done one chromosome at a time so that the individual effects of each chromosome and its interaction with the others can be studied for a quantitative trait. Before selection began, chromosomes X and II contributed to positive geotaxis, while chromosome III influenced negative geotaxis. Selection for positive geotaxis had little effect on the influence of chromosome II, but it increased the positive effect of chromosome X and changed the negative effect of chromosome III to slightly positive. Similar changes in the effects of the chromosomes occurred in the line selected for negative geotaxis. This analysis indicates that loci on all three chromosomes affect geotaxis. Continuing with these techniques, it would be possible to locate precise regions on chromosomes that have precise effects on geotaxis (Ehrman and Parsons 1976). This is an exciting concept, and it is made possible by the previous extensive exploration of *Drosophila* genetics.

Other Behaviors

Although geotaxis has been most completely analyzed, selective breeding has been done for several other *Drosophila* behaviors: chemotaxis (Becker 1970), phototaxis (Hirsch and Boudreau 1958), activity (Connolly 1966), and mating speed (MacBean and Parsons 1967; Manning, 1961, 1963, 1968). The Manning studies on mating speed were actually the first to use the design of replicated lines with an unselected control, the design later used by DeFries and others with mice.

Conclusion

Several points emerging from this review are worth reiterating. First, *behavioral measurement* is extremely important to the behavioral selection study; issues of reliability and validity of measurement are critical. Second, the *genetic design* is also critical. Replicate lines are quite advantageous because they allow the investigator to draw much stronger conclusions than a single set of lines would. Current thought also favors the minimizing of inbreeding. Third, most selection studies provide evidence of the *polygenic* control of behavior. Finally, very elegant and precise analyses are possible based on selective breeding, as demonstrated by the *Drosophila* studies.

References

Becker, H.J. The genetics of chemotaxis in *Drosophila melanogaster*: Selection for repellant insensitivity. *Mol Gen Genet*, 107:194-200, 1970.

Bignami, G. Selection for high rates and low rates of avoidance conditioning in the rat. *Anim Behav*, 13:221-227, 1965.

Blizard, D.A., and Chai, C.K. Behavioral studies in mice selectively bred for differences in thyroid function. *Behav Genet*, 2:301-309, 1972.

Bovet, D.; Bovet-Nitti, F.; and Oliverio, A. Genetic aspects of learning and memory in mice. *Science*, 163:139-149, 1969.

Broadhurst, P.L. Experiments in psychogenetics: Applications of biometrical genetics to the inheritance of behavior. In: Eysenck, H.J., ed. *Experiments in Personality. Vol. 1. Psychogenetics and Psychopharmacology.* London: Routledge and Kegan Paul, 1960. pp. 1-102.

Broadhurst, P.L. The Maudsley reactive and nonreactive strains of rats: A survey. *Behav Genet*, 5:299-319, 1975.

Chen, C.S., and Fuller, J.L. Selection for genetically determined or priming-induced audiogenic seizure susceptibility in mice. *Behav Genet*, 5:91-92, 1975. (Abstr.)

Collins, R.-A. Aggression in mice selectively bred for brain weight. *Behav Genet*, 1:169-171, 1970.

Connolly, K. Locomotor activity in *Drosophila*. II. Selection for active and inactive strains. *Anim Behav*, 14:444-449, 1966.

Craig, J.V.; Ortman, L.L.; and Guhl, A.M. Genetic selection for social dominance ability in chickens. *Anim Behav*, 13:114-131, 1965.

Cunningham, D.L., and Siegel, P.B. Response to bidirectional and reverse selection for mating behavior in Japanese quail, *Coturnix coturnix japonica. Behav Genet*, 8:387-398, 1978.

Deckard, B.S.; Tepper, J.M.; and Schlesinger, K. Selective breeding for acoustic priming. *Behav Genet*, 6:375-384, 1976.

DeFries, J.C., and Hegmann, J.P. Genetic analysis of open-field behavior. In: Lindzey, G., and Thiessen, D.D., eds. *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype*. New York: Appleton-Century-Crofts, 1970. pp. 23-56.

DeFries, J.C.; Gervais, M.C.; and Thomas, E.A. Response to 30 generations of selection for open-field activity in laboratory mice. *Behav Genet*, 8:3-14, 1978.

DeFries, J.C.; Wilson, J.R.; and McClearn, G.E. Open-field behavior in mice: Selection response and situational generality. *Behav Genet*, 1:195-212, 1970.

Dobzhansky, T., and Spassky, B. Selection for geotaxis in monomorphic and polymorphic populations of *Drosophila pseudoobscura. Proc Natl Acad Sci*, 48:1704-1712, 1962.

Ebert, P.D., and Hyde, J.S. Selection for agonistic behavior in wild female *Mus musculus. Behav Genet*, 6:291-302, 1976.

Ehrman, L., and Parsons, P.A. *The Genetics of Behavior*. Sunderland, Mass.: Sinauer, 1976.

Elias, J.W.; Elias, M.F.; and Schlager, G. Aggressive social interaction in mice genetically selected for blood pressure extremes. *Behav Biol*, 13:155-166, 1975.

Elias, M.F. Differences in spatial discrimination reversal learning for mice genetically selected for high brain weight and unselected controls. *Percept Mot Skills*, 28:707-712, 1969.

Elias, M.F., and Schlager, G. Discrimination learning in mice genetically selected for high and low blood pressure: Initial findings and methodological implications. *Physiol Behav*, 13:261-267, 1974.

Erlenmeyer-Kimling, L.; Hirsch, J.; and Weiss, J.M. Studies in experimental behavior genetics. III. Selection and hybridization analyses of individual differences in the sign of geotaxis. *J Comp Physiol Psychol*, 55:722-731, 1962.

Festing, M.F.W. Water escape learning in mice. I. Strain differences and biometrical considerations. *Behav Genet*, 3:13-24, 1973*a*.

Festing, M.F.W. Water escape learning in mice. II. Replicated selection for increased learning speed. *Behav Genet*, 3:25-36, 1973*b*.

Festing, M.F.W. Water escape learning in mice. III. A diallel study. *Behav Genet*, 4:111-124, 1974.

Frings, H., and Frings, M. The production of stocks of albino mice with predictable susceptibilities to audiogenic seizures. *Behavior*, 5:305-319, 1953.

Fuller, J.L., and Thompson, W.R. *Behavior Genetics*. New York: Wiley, 1960.

Ginsburg, B.E. Genetic parameters in behavioral research. In: Hirsch, J., ed. *Behavior-Genetic Analysis*. New York: McGraw-Hill, 1967. pp. 135-153.

Graves, H.B., and Siegel, P.B. Bidirectional selection for responses of *Gallus domesticus* chicks to an imprinting situation. *Anim Behav*, 17:683-691, 1969.

Griffiths, W.J. Transmission of convulsions in the white rat. *J Comp Psychol*, 34:263-277, 1942.

Guhl, A.M.; Craig, J.V.; and Mueller, C.D. Selective breeding for aggressiveness in chickens. *Poult Sci*, 39:970-980, 1960.

Halcomb, R.A.; Hegmann, J.P.; and DeFries, J.C. Open-field behavior in mice: A diallel analysis of selected lines. *Behav Genet*, 5:217-231, 1975.

Hall, C.S. The inheritance of emotionality. *Sigma Xi Q*, 26:17-27, 1938.

Hall, C.S. The genetics of behavior. In: Stevens, S.S., ed. *Handbook of Experimental Psychology*. New York: Wiley, 1951. pp. 304-329.

Heron, W.T. The inheritance of maze-learning ability in rats. *J Comp Psychol*, 19:77-89, 1935.

Heron, W.T. The inheritance of brightness and dullness in maze learning ability in the rat. *J Genet Psychol*, 59:41-49, 1941.

Hewitt, J.K.; Fulker, D.W.; and DeFries, J.C. Open-field behavior in mice: Generality of results from a diallel analysis of replicate selected lines. *Behav Genet*, 7:441-446, 1977.

Hirsch, J. Behavior genetics and individuality understood. *Science*, 142:1436-1442, 1963.

Hirsch, J., and Boudreau, J. Studies in experimental behavior genetics. The heritability of phototaxis in a population of *Drosophila melanogaster*. *J Comp Physiol Psychol*, 51:647-651, 1958.

Hurnik, J.F.; Bailey, E.D.; and Jerome, F.N. Selection for divergent lines of mice based on their performance in a T-maze. *Behav Genet*, 3:45-55, 1973.

Hyde, J.S., and Ebert, P.D. Correlated response in selection for aggressiveness in female mice. I. Male aggressiveness. *Behav Genet*, 6:421-428, 1976.

Hyde, J.S., and Sawyer, T.F. Estrous cycle fluctuation in aggressiveness of house mice. *Horm Behav*, 9:290-295, 1978.

Hyde, J.S., and Sawyer, T.F. Selection for agonistic behavior in wild female mice. *Behav Genet*, 10:349-359, 1980.

Krech, D.; Rosenzweig, M.R.; and Bennett, E.L. Dimensions of discrimination and level of cholinesterase activity in the cerebral cortex of the rat. *J Comp Physiol Psychol*, 49:261-268, 1956.

Kuppaswany, B. Laws of heredity in relation to general mental ability. *J Gen Psychol*, 36:29-43, 1947.

Lagerspetz, K. Studies on the aggressive behavior of mice. *Ann Acad Sci Fenn (B)*, 131:1-131, 1964.

Lagerspetz, K.M.J., and Lagerspetz, K.Y.H. Changes in the aggressiveness of mice resulting from selective breeding, learning, and social isolation. *Scand J Psychol*, 12:241-248, 1971.

Lagerspetz, K.Y.H.; Tirri, R.; and Lagerspetz, K.M.J. Neurochemical and endocrinological studies of mice selectively bred for aggressiveness. *Scand J Psychol*, 9:157-160, 1968.

MacBean, I.T., and Parsons, P.A. Directional selection for duration of copulation in *Drosophila melanogaster*. *Genetics*, 56:233-239, 1967.

Maier, N.R.F. Studies of abnormal behavior in the rat. XIV. Strain differences in the inheritance of susceptibility to convulsions. *J Comp Psychol*, 35:327-335, 1943.

Manning, A. The effects of artificial selection for mating speed in *Drosophila melanogaster*. *Anim Behav*, 9:82-92, 1961.

Manning, A. Selection for mating speed in *Drosophila melanogaster* based on the behavior of one sex. *Anim Behav*, 11:116-120, 1963.

Manning, A. The effects of artificial selection for slow mating in *D. simulans*. *Anim Behav*, 16:108-113, 1968.

McClearn, G.E. The inheritance of behavior. In: Postman, L., ed. *Psychology in the Making*. New York: Knopf, 1962. pp. 144-252.

Nachman, M. The inheritance of saccharin preference. *J Comp Physiol Psychol*, 52:451-457, 1959.

Rhine, J.B., and McDougall, W. Third report on a Lamarckian experiment. *Br J Psychol*, 24:213-235, 1933.

Rosenzweig, M.R.; Krech, D.; Bennett, E.L.; and Longueil, C. "Strain Differences of Rats in Behavior and Brain Chemistry." Paper presented at the annual meeting of the American Psychological Association, Washington, D.C., September 1958.

Rundquist, E.A. The inheritance of spontaneous activity in rats. *J Comp Psychol*, 16:415-438, 1933.

Searle, L.V. The organization of hereditary maze-brightness and maze-dullness. *Genet Psychol Monogr*, 39:279-325, 1949.

Siegel, P.B. Genetics of behavior: Selection for mating ability in chickens. *Genetics*, 51:1269-1277, 1965.

Smith, R.H. Selection for shuttle avoidance in wild *Mus musculus*. *Behav Genet*, 8:269-274, 1978.

Thompson, W.R. The inheritance and development of intelligence. *Proc Assoc Res Nervous Mental Disease*, 33:209-231, 1954.

Tolman, E.C. The inheritance of maze-learning ability in rats. *J Comp Psychol*, 4:1-18, 1924.

Tryon, R.C. Genetic differences in maze-learning ability in rats. *39th Yearbook of the Natl Soc Stud Education*, 39(1):111-119, 1940.

Tryon, R.C. Individual differences. In: Moss, F.A., ed. *Comparative Psychology*. New York: Prentice-Hall, 1942.

van Abeelen, J.H.F. Genetics of rearing behavior in mice. *Behav Genet*, 1:71-76, 1970.

van Abeelen, J.H.F.; van der Kroon, P.H.W.; and Bekkers, M.F.J. Mice selected for rearing behavior: Some physiological variables. *Behav Genet*, 3:85-90, 1973.

Watanabe, T.K., and Anderson, W.W. Selection for geotaxis in *Drosophila melanogaster*: Heritability, degree of dominance, and correlated responses to selection. *Behav Genet*, 6:71-86, 1976.

Wimer, C., and Prater, L. Some behavioral differences in mice genetically selected for high and low brain weight. *Psychol Rep*, 19:675-681, 1966.

Wimer, C.; Roderick, T.H.; and Wimer, R.E. Supplementary report: Behavioral differences in mice genetically selected for brain weight. *Psychol Rep*, 25:363-368, 1969.

SECTION II: SELECTIVE BREEDING FOR ALCOHOL-RELATED PHENOTYPES

Introduction

Gerald E. McClearn

This section describes the principal programs of selective breeding for alcohol-related phenotypes that have been done to date. All of them were successful in generating divergent lines. Thus they all show, retrospectively, that heritability for the phenotype in the foundation population was nonzero. A long-term selection study is, of course, a very expensive way to demonstrate that fact, and many alternative, less expensive procedures would be available were that the only objective. Of greater importance is the fact that, once generated, these lines become available as test beds for hypotheses concerning the mechanisms—pharmacological, biochemical, physiological, or behavioral—that underlie the phenotypic difference.

In the case of the older programs in Finland (Eriksson and Rusi) and in Colorado (McClearn and Kakihana), sufficient information on the correlates and mechanisms has accumulated to warrant separate chapters describing the origins and the subsequent research. In the case of the more recent programs in Indiana (Li, Lumeng, McBride, and Waller) and at Rutgers (Bass and Lester), the origins of the selected lines and the subsequent research performed with them are concisely combined in individual chapters. Here, as in the behavioral studies reviewed in the first section, and for many of the same reasons, we see that the designs were often less than the optimal design recommended in section IV. Once again, however, we observe that the results have been substantial in spite of design shortcomings. In many cases, there is essentially no overlap between distributions of measures for contemporary generations of the upward and downward selected lines.

One way of viewing the investigations into the pharmacological, biochemical, physiological, and behavioral mechanisms that determine the line differences is to regard them as investigations of *correlated responses* to selection. If selection has been sufficiently intense for sufficiently long, then we should have sifted all of the available + alleles in the gene pool with which we began into the upward selected (high) line and all of the - alleles into the downward selected (low) line—ignoring, for immediate purposes, the possibility of selection for heterozygotes.

Let us assume that there exist 5 loci that influence the phenotype of concern, e.g., alcohol preference, and that we identify these loci as *A* through *E*. (It is possible that 50 or 500 loci might be a more realistic assumption, but we shall consider 5 for convenience.) Let us assume further that our foundation stock is segregating for all of these loci. Thus, there will be *AA*, *Aa* and *aa* genotypes; *BB*, *Bb* and *bb* genotypes; etc. Furthermore, let us assume that the capital letters signify + alleles and the lowercase letters signify - alleles. Gradually, as selection proceeds, the + and - alleles will be maneuvered into the high line and the low line, respectively. At the limits of selection response, therefore, all animals within the high line will be *AABBCCDDEE*, while all animals in the low line will be *aabbccddee*.

Locus A might influence our selected trait of alcohol preference through some taste mechanism; locus B through cytosolic alcohol dehydrogenase (ADH) activity; locus C through the MEOS activity; locus D through some feature of the limbic system neuron's sensitivity to alcohol; etc. We probably will not know any of this in advance; we will perform the selection and then examine hypothesized mechanisms. We will then discover that the taste mechanism, the ADH activity, the MEOS activity, and so on, are implicated. In genetic parlance, they are "correlated responses" to the selection. Pharmacologically, they are part of the causal nexus.

Now, if we avoid deliberate inbreeding, and if our sample size is sufficiently great so that inadvertent inbreeding is minimized, then little "drift" of alleles unrelated to the selected phenotype should occur. That is, for the nonrelevant loci—say, *F* through *Z*—there will have been no directional influences resulting in the congregation of the + alleles of any of these loci into one line or the other. Thus, we can imagine high-line animals of the following genotypes:

AABBCCDDEEFFGGHHIIJJKK. . .
AABBCCDDEEFFGghhiiJjKk. . .
AABBCCDDEEffggHhIijjkk. . .
AABBCCDDEEffgghhiijjkk. . .
and so on.

Similarly, we can imagine low-line animals with the following genotypes:

aabbccddeeFFGGHHIIJJKK. . .
aabbccddeeFFGghhiiJjKk. . .
aabbccddeeffggHhIijjkk. . .
aabbccddeeffgghhiijjkk. . .
and so on.

The only allelic combination unique to the high line is *AABBCCDDEE*; for the low line, the combination *aabbccddee* is unique. Any combination of alleles at the other loci can occur in either line, and, thus, traits influenced by these other loci (and not influenced by loci *A* through *E*) on the average will not differ between the lines.

This situation is in strong contrast to the rather weak evidence that can be derived from comparisons of inbred strains with regard to two or more phenotypes. As has been argued elsewhere (McClearn 1979), the fact that inbred C57BL mice are high in both alcohol preference and alcohol dehydrogenase activity, whereas DBA mice are low in both of these characters (Rodgers and McClearn 1962), is not particularly informative because the process of inbreeding in effect causes fixation at all loci. Because the particular alleles fixed within any one strain are random, any two strains will be expected to differ with regard to an indefinitely large number of phenotypes that may be causally unrelated to each other.

To the extent that systematic inbreeding is practiced or sample size restriction leads to inbreeding, in effect if not in intent, the ideal case of the selected lines as described above is weakened and they become more like inbreds in this respect. However, there is a great deal of difference between systematic sib mating for 20 or more generations and the inbreeding that occurs as a result of a small number of families in either or both of two selected lines or because of a bottleneck caused by too few families in one generation or for any other reason. The proof of the pudding is in the eating, of course, but it would seem that the selected lines described here, while less than ideal, may be very useful in identifying causal mechanisms through comparisons of mean values of the lines on the properties in question. In any case, the strategy of using systematically heterogeneous stocks or of crossing lines to get an F_1 and then intercrossing F_1's to get an F_2 generation can give the investigator the powerful possibility of correlational analysis (McClearn 1979).

Another issue that arises in interpreting research on selected lines concerns the consistency of findings with respect to correlated characters. Imagine, for example, a selection study that has successfully generated lines of mice (or rats) that show high or low preference for alcohol. Subsequent research shows the high line to be characterized by more of trait *Y* than the low line. Need we expect this same result when we compare high- and low-preference animals generated by some other selection program? Not at all. rat (or mouse), just as there are undoubtedly many complex factors that make for human avidity for the drug. The pattern of causal influences that differentiates a high line and a low line derived from one particular foundation stock, with its particular gene pool, need not be the same as that which differentiates lines derived from another foundation stock with its different gene pool.

For example, let us consider again our five hypothetical loci influencing alcohol preference. Now, in a particular heterogeneous stock that might be chosen as the foundation population, one locus might happen to be fixed; that is, every animal within that stock might be *AA*. Clearly, selection cannot do anything with such a locus unless and until the very rare event of a mutation occurs at that locus in one of the animals in the study. If the foundation stock is segregating at the other loci, then we can still have *bb*, *Bb*, *BB* animals; *cc*, *Cc*, *CC* animals; etc.; and selection should gradually yield the following genotypes for the five loci: *AABBCCDDEE* for the high line, and *AAbbccddee* for the low line. It is clear that whatever differences in alcohol preference exist between the high and low lines cannot be due to any influence of the *A* locus. Thus, to continue our earlier suppositions, we would find no evidence of the taste variable in this stock. Another foundation population might be segregating for the *A* locus and for the *C*, *D*, and *E* loci, but be fixed for *B*—say, as the homozygote *bb*. Then the high and low lines derived from this foundation stock will show no difference in ADH activity (again, unless ADH activity were also influenced by the *A*, *C*, *D*, or *E* locus).

To reiterate, if two lines have been selected for trait *X* and are found to differ also with respect to trait *Y* (in the absence of inbreeding, as described above), one can infer that trait *Y* can be part of the causal network that determines trait *X*. If high and low lines with respect to trait *X* are found not to differ in trait *Y* in some other selection study, this information reveals that trait *Y* is not an *essential* part of the causal network. If trait *Y* is consistently found to differ between lines differing in *X*, one might gradually build confidence that trait *Y* is a *necessary* part of that network.

Naturally, there are other approaches than the study of correlated characters to elucidating causal mechanisms. Intervention experiments can seek to alter the levels of trait *Y*, pharmacologically or by some other means, and then observe the effects of trait *X*, for example. Insights into causal mechanisms distinguishing selected lines might thus be sought by denervation of taste systems, by administration of antagonists, by selective ablation, or by stimulation of the nervous system, among other procedures. The important point is that the lines generated by selective breeding constitute efficient tools for the study of the basic features underlying the phenotypes for which they differ.

References

McClearn, G.E. Influence of genetic variables on means, variances, and covariances in behavioral responses to toxicological and pharmacological substances. *J Toxicol Environ Health,* 5:145-156, 1979.

Rodgers, D.A., and McClearn, G.E. Alcohol preference of mice. In: Bliss, E.L., ed. *Roots of Behavior.* New York: Hoeber, 1962. pp. 68-95.

Finnish Selection Studies on Alcohol-Related Behaviors: General Outline

Kalervo Eriksson and Maija Rusi

The first and most important question is, Why should we try to produce specially selected animal strains for alcohol research? For a correct understanding of the etiology of alcoholism and the basic mechanisms behind drinking behavior, intoxication, tolerance, and physical dependence, it is very important to map the inherited individual differences and the joint genetic-environmental interactions of alcohol-related traits. Using laboratory mice and rats as models, one can try to elucidate the biological factors determining alcohol-related behaviors and reactions.

Much work has been done relating voluntary alcohol consumption with certain behavioral traits or metabolic characteristics of inbred mice. However, the inbred strains used have been genetically selected for traits other than their responses to alcohol, so the correlations found between biochemical and behavioral properties may have been produced quite coincidentally. Consequently, inbred mice are not particularly suitable for research on the etiology of drinking.

In contrast to inbreeding, selective outbreeding is a directional process. Thus, a successful genetic selection by outbreeding may give retrospective evidence for the existence of additive genetic variance with respect to the trait in the base population. With this technique there is also a much greater probability of finding metabolic and behavioral factors truly related to subjects' responses to ethanol.

For these reasons, in 1963, a selective outbreeding of high- and low-line rat strains for their voluntary alcohol consumption was begun in the Research Laboratories of the State Alcohol Monopoly of Finland (Alko). The rat strain with high voluntary ethanol consumption has been called AA (Alko, alcohol strain) and the strain with low alcohol intake has been called ANA (Alko, nonalcohol strain). The progress of this work was described and discussed earlier (K. Eriksson 1968, 1969, 1971, 1972; K. Eriksson and Närhi 1973). A large amount of comparative research also has been done with these animal strains (this research is presented in detail in the chapter by C.J.P. Eriksson).

The purpose of the genetic selection was to produce one albino rat strain that voluntarily drinks physiologically effective amounts of ethanol solution and another that avoids alcohol as completely as possible. The drinking behavior of both of these strains now seems to have stabilized and reached its final form. So it seems appropriate at this time to examine the differences between strains and to consider especially the problems of standardizing and determining the phenotype.

Voluntary drinking behavior is only one alcohol-related behavior. Some behaviors may be independent of drinking behavior, while others are related to it. In humans, various signs and syndromes are found jointly with excessive drinking; of these, deviations in acute intoxication and tolerance are among the most important. Consequently, we have tested whether the AA and ANA strains developed for different drinking behavior also vary in their susceptibility to intoxication or in their innate tolerance to ethanol given i.p., when measured with both the tilting plane test (Nikander and Pekkanen 1977) and the sleeping time test (Rusi et al. 1977). Clear strain differences have been found, with the AA rats having a higher tolerance for acute ethanol intoxication. Compared with that of ANA rats, the motor coordination of AA rats was found to be less affected by isopropanol, t-butanol, and barbital, as well as by ethanol (Malila 1978). These findings suggest the existence of a highly inherited mechanism behind innate tolerance. They also suggest that inherited drinking behavior is involved with innate tolerance. Consequently, it seemed of high value for alcohol research to produce animal strains specially selected for high or low innate ethanol tolerance. Thus, in 1975, we started selective outbreeding with rats for differential susceptibility to intoxication with two different doses of ethanol. (A short preliminary report of this selection study was published earlier—Rusi et al. 1977.) The new rat strains are called AT (alcohol tolerant strain) and ANT (alcohol nontolerant strain). We have now reached the F_7 generation, where relatively high strain differences are found, and we feel that we have probably arrived at the most suitable and practical phenotype determination. It seems appropriate to describe these strain differences in innate tolerance and their relation to drinking behavior.

Genetic Origin of the AA and ANA Rat Strains

The animals used in this genetic selection work were from a conventionally housed ordinary albino rat stock of Wistar origin raised in our laboratory since 1954. At an early stage the original stock was also crossbred with some Sprague-Dawley albinos.

Before the selection began, the basic population was systematically bred for more than 20 generations, so that as distant relatives as possible were used for reproduction. The number in the colony was 500-700, and breeding animals were selected from a large number of families (10-15) in every generation. For that reason, there was very large individual variation in voluntary alcohol intake when selective outbreeding was started (K. Eriksson 1969).

To avoid restriction of individual variation at a given level, sib mating was intentionally avoided at first. The first 12 outbred generations constituted two separate lines, and the selection of respective inbred lines was started from four families of both strains in the 12th generation. A short description of these inbred animals was published earlier showing typical strain characteristics (K. Eriksson 1972). These inbred lines are still maintained in our laboratory, but they will not be discussed here.

Meanwhile, outbreeding was continued. The alcohol drinking behavior was tested in every generation and then about 20-25 percent of the population were used for subsequent breeding. The population size of the generations in both strains has varied between 80 and 140.

Standardization of the Phenotype in the AA and ANA Strains with Special Comments to Selection Goal

Housing

The housing, feeding, and complete technique for measuring alcohol intake have been described in detail (K. Eriksson 1968, 1969, 1971). The light and dark cycle is 12 hours, relative humidity is held between 45 and 55 percent, and the environmental temperature is kept between 22° and 26°C. The first 18 generations of subjects were raised on our own laboratory food (see K. Eriksson 1969). From the F_{19}generation on, we have used the standard food produced by Astra-Ewos®, Södertälje, Sweden.

Drinking Behavior

The subjects are kept in individual cages while their alcohol consumption is tested. Testing begins when they are 3 months old. For the first 10 days, 10 percent (v/v) ethanol solution is provided as the sole fluid. After this "habituation period," they can choose between tap water and 10 percent (v/v) ethanol solution and receive standard food ad libitum for 3 weeks. The positions of the drinking bottles are reversed weekly. For the analysis the mean consumption figures of the third week are used as a measure of the phenotype.

Phenotypic Measures

The voluntary ethanol intake is usually expressed as the alcohol preference figure, i.e., the amount of ethanol solution consumed as a percentage of total fluid consumption or as the related E/T value. These figures are often used for determining the phenotype of drinking behavior in genetic research. This phenotypic score depends on the total fluid consumption and the liquid requirement of the subject (K. Eriksson 1969). It also depends on the humidity of the environment, the water content of the food, etc. Furthermore, the alcohol preference score is a relative value, which should be subjected to arc-sine transformation before it can be used in biometrical analyses based on the variance ratio, a procedure which, however, usually has not been done (Brewster 1969). Without doubt, two different strains could be produced by using only the preference score as a measure of ethanol intake: one strain having a very small ethanol intake but a high fluid intake and the other consuming much ethanol but very little total fluid. The relevance of such strains would be questionable.

Similar difficulties are encountered with the other measures. One possibility is to calculate the ethanol consumption in relation to body weight. It has been demonstrated experimentally (K. Eriksson 1969) that rat strains tend to drink roughly the same absolute amount of alcohol per unit of body weight over a certain concentration range, i.e., 5-10 percent (v/v), with the amount of alcohol consumed differing between individuals and strains. This value can, therefore, be used relatively well for comparing the drinking that occurs with different concentrations within this range. As might be expected, however, the amount does not remain constant at extremely high or low concentrations. The amount of alcohol consumed per unit of body weight (g/kg) also reflects relatively well the pharmacological role of ethanol. During a long period of genetic selection, however, this measure of phenotype can also lead to artifactual results. This phenotypic measure selects subjects not only on the basis of their ethanol drinking behavior but also on the basis of body weight. For instance, in the early phases of developing the AA and ANA strains, we measured both the preference score and the ethanol intake per body weight, but weighted the latter more heavily in the selection. As a result, we got AA rats with high ethanol intake but also with lighter body weight, and ANA rats with low ethanol intake but also heavier body weight (K. Eriksson 1968, 1972). The strain difference in body weight was also related to differences in total energy intake (K. Eriksson and Närhi 1973).

We needed additional measures. If we want to consider the energy content of ethanol or the metabolic role of ethanol, it seems reasonable to express ethanol intake in relation to total caloric intake, i.e., the preference for alcohol calories (energy from alcohol as a percentage of total energy intake). In a normal self-selection situation, there is a very high positive correlation, 0.97, between this measure and the alcohol intake per unit of body weight (K. Eriksson 1969), so they appear equal from a statistical point of view.

By the F_{19} generation we found striking differences in body weight and in energy intake between the AA and ANA rats (K. Eriksson and Närhi 1973). Consequently, from this generation we selected primarily on the basis of caloric preference score. The result was that the strain difference in energy intake disappeared, while differences in body weight were reversed (table 1), with the AA rats in the F_{29} generation being significantly heavier than the ANA rats. Clearly this third measure can also strongly bias the selection results. Subsequently, we have combined the three measures with approximately equal weights when selecting animals for breeding.

Table 1. Mean Body Weight (G) in the AA and ANA Rats of the F_{20} and F_{29} Generations at the Age of 3.5 Months (Mean ± S.D.)

Strain	Sex	F_{20} Generation	F_{29} Generation
AA	M	286 ± 22 (19)[1]	265 ±29 (33)
		N.S.[2]	$p < 0.0001$
ANA	M	297 ± 33 (9)	230 ±29 (21)
AA	F	179 ± 11 (17)	174 ± 13 (38)
		N.S.	$p < 0.0001$
ANA	F	185 ± 9.8 (10)	154 ± 15 (21)

[1] Number of rats is given in parentheses

[2] N.S. = Not Significant

The present drinking behavior of AA and ANA rats of the F_{32} generation is shown in table 2. The AA rats have a significantly higher preference score than the ANA rats. The strain difference in alcohol intake is as striking when calculated as absolute ethanol intake per body weight.

Table 2. Voluntary Ethanol Consumption During the Self-Selection Period in the F_{32} Generation of AA and ANA Rats (Mean ± S.D.)

Strain	Sex	*N*	Preference Ratio (Percentage from Total Fuids)	Alcohol Intake (g/Kg/day)	Energy from Ethanol (Percentage of Total Energy)
AA	M	45	59.2 ± 19.1	4.82 ± 1.71	16.4 ± 5.3
			$p < 0.001$	$p < 0.001$	$p < 0.001$
ANA	M	29	17.0 ± 11.2	1.06 ± 0.62	4.2 ± 2.6
AA	F	48	65.1 ± 20.2	6.55 ± 2.33	18.3 ± 5.7
			$p < 0.001$	$p < 0.001$	$p < 0.001$
ANA	F	29	11.2 ± 12.0	0.83 ± 0.93	3.0 ± 3.4

The total ethanol intake is lower in these strains now than was presented earlier (K. Eriksson 1969, 1971, 1972), apparently as a result of the change in diet started in the F_{18} generation.

Table 3 shows the mean body weight, total energy intake, and total fluid intake of AA and ANA rats in the F_{32} generation. The difference in body weight is not statistically significant, but it is in the same direction as that found in the F_{29} generation (table 1). The total energy intake and total fluid intake of the AA rats are significantly higher than for the ANA rats, reflecting the response to the selection pressure occurring after the phenotypic determination was changed.

Table 3. Mean Body Weight, Total Energy Intake, and Total Fluid Intake of the F_{32} Generation of the AA and ANA Rats During the 3-Weeks Self-Selection Period (Mean ± S.D.)

Strain	Sex	*N*	Body Weight[1]	Total Energy[2]	Total Fluids[3]
AA	M	45	307 ± 17	873 ± 97	103 ± 17
			N.S.	$p < 0.001$	$p < 0.001$
ANA	M	29	296 ± 28	757 ± 42	81 ± 10
AA	F	48	193 ± 14	1061 ± 217	126 ± 22
			N.S.	$p < 0.001$	$p < 0.001$
ANA	F	29	186 ± 18	865 ± 219	98 ± 20

[1] Body Weight is measured in grams.

[2] Energy Intake measured by kJ/kg body weight/day

[3] Fluid Intake measured by ml/kg body weight/day

Genetic Origin of the AT and ANT Rats

The production of the basic population was started in 1973 in the Physiological Department of the Research Laboratories of the State Alcohol Monopoly (Alko). A crossbreeding program was instigated to produce a maximally heterogeneous laboratory rat colony and also to produce subjects with a relatively high resistance to normal laboratory rat diseases. We crossbred Wistar, Sprague-Dawley, and Long-Evans originated animals, which are the most commonly used rat strains in laboratory studies. We can assume that the resulting mixed strain contains a great deal of the genetic variation present in the total population of laboratory rats. If this is true, the results obtained from these animals are more generalizable to all rat populations than ones obtained from any single strain. *Cross-breeding analysis of basic population (mixed strain)*:

P: Albino rats from the Helsinki Zoo (Korkeasaari) (origin unknown) were crossbred with AA rats of Wistar origin, F_{22} females. The number of breeding pairs was 6.

F_1: Males of the ANA strain from the F_{23} were crossbred with P females. The number of breeding pairs was 11.

F_2 : Sprague-Dawley males (Anticimex, Sweden) were crossbred with mixed strain F_1 females. The number of breeding pairs was 10.

F_3 : Reciprocal crossbreeding of the F_2 animals with Long-Evans hooded rats (Simonsen Laboratories, Inc., Calif.). The number of breeding pairs was 15.

F_4 : Randomly selected males and females of the F_3 mixed population were bred. The number of breeding pairs was 18. Since then, in every generation intentional crossbreeding was maintained, avoiding the breeding of relatives.

The starting population for the selection study of the AT and ANT rats was the F_6 generation of mixed strain rats. Many things argue for the superiority of the resulting mixed strain. It is more suitable for genetic selection work because it is the result of crossbreeding many rat strains, and it is, therefore, in this way genetically heterogenic. The enzyme polymorphism of the mixed strain is, however, quite low compared with wild populations and is in the same class as that of the outbred strains of laboratory rats (K. Eriksson et al. 1976). The low number of polymorphic loci indicates that the three laboratory rat strains most commonly used in breeding programs are quite similar.

The reproductive ability of the mixed strain is very good (table 4). This reproductive ability has been measured by counting the number of pups born/litter and by counting the fertility index for the females using the formula number of pregnant females/number of females mated × 100 (Curley and Pelas 1969).

Table 4. Mean Litter Sizes (±S.D.) and Fertility Indexes for the Female Rat of the Different Strains in the Research Laboratories of State Alcohol Monopoly of Finland

Strain	*N*	Number of Pups/Litter	Fertility Index
AA $_{F\,31}$	19	10.3 ± 1.8	76.7
ANA $_{F\,31}$	11	7.2 ± 1.5	75.0
Long-Evans $_{F\,7}$	11	9.2 ± 2.5	78.6
Mixed Strain $_{F\,9}$	15	11.1 ± 2.5	79.2
AT $_{F\,7}$	7	13.0 ± 2.0	100.0
ANT $_{F\,7}$	7	11.6 ± 2.7	100.0

[1] Basic population for AT and ANT rats.

The rats of the mixed strain grow well; their body sizes are in the same class as Long-Evans rats, and they are much heavier than the AA and ANA rats. Moreover, the mixed strain has proved to be very resistant to the lung infection caused by *Mycoplasma pulmonis*. This disease has been a difficult problem in our laboratory for many years (it is a common problem in most conventional animal facilities). Especially on long-term tests, the resistance of the animals tends to weaken, and they easily are infected with respiratory diseases. The health of the mixed strain rats has been examined by regularly sending rats to the State Veterinary Institute for investigation; their health has been found to be good. Intestinal worms, congenital kidney disorders, and specific bacterial infection have not been found, and mild symptoms of *Mycoplasma pulmonis* infection in the lungs have been found in only a few animals.

Standardization of the Phenotype in AT and ANT Rats

The purpose of this study was to produce two new rat strains, one with high susceptibility to acute ethanol intoxication and one with low susceptibility to ethanol intoxication after an acute moderate dose of ethanol. The degree of intoxication was to be tested at the same level of blood alcohol. Because this research was envisioned as an ongoing program, the procedures used in testing and breeding were constantly assessed and modified.

Housing

The rats were maintained under standard laboratory conditions on a 12-hour day-night cycle at 22-26°C. Standard food (Astra-Ewos®, Ab Astra, Södertälje, Sweden) and water were available ad libitum. At weaning (21-25 days of age) the rats were separated from their mother and housed in plastic cages, each sex being housed separately, four rats per cage. About 2 weeks before the beginning of the test period the rats were moved into stainless steel laboratory cages, seven or eight rats per cage.

Test Procedure

The rats were food deprived 4 hours before ethanol injection. Three tests of alcohol sensitivity were used: open field, tilting plane, and rotarod (they are described below). The control values without ethanol in the tilting plane and in the rotarod were taken 3 hours before injection of ethanol. In generation F_0 - F_4 these were taken without saline injection, but from the F_5 generation on, they were taken after saline injection (0.5 ml/100 gb body weight). The ethanol doses were as follows: in the open field, 1.2 g/kg body weight; in the tilting plane, 2.0 g/kg body weight; and in the rotarod, 1.5 g/kg body weight. The ethanol was given as a 12 percent (w/v) solution in saline and administered intraperitoneally. The temperature of the injected ethanol solution was 37°C. Ethanol was given 30 minutes before testing in all cases except with the F_6 generation, to which it was given 45 minutes before testing. All the tests were carried out under the same conditions, and they were performed blind at the same time of day, between 11:30 a.m. and 2:00 p.m. The time between the different tests was at least 7 days. Blood alcohol determinations were made at the end of each test session.

Following testing of the entire generation, rats were selected for breeding and paired. Selection of breeders was based primarily on the impairment of performance on the tilting plane test, but rotarod performance and blood alcohol levels were also considered. The general procedure is summarized in table 5.

Blood Alcohol Determination

A blood sample of 0.1 milliliters was taken from the tip of the rat's tail and pipetted into 0.9 milliliters of ice-cold distilled water. The ethanol levels in the tail blood were measured with head space gas chromatography (C. Eriksson 1973; C. Eriksson et al. 1977).

Tests for Tolerance and Intoxication

Tilting Plane.

Motor coordination of the animals was measured by the standardized tilting plane test in a motorized form. (The tilting plane is described in greater detail by Arvola et al. 1958). During a 5-second

Table 5. Summary of Testing Procedures Used in Developing the Alcohol Tolerant (AT) and Alcohol NonTolerant (ANT) Rat Strains

Generation	N	Age at Testing (days)	Test[1]	Dose of Ethanol (g/kg b.w.)	Number of Breeding Pairs/Strains
F_0	80	90-105	Open-field	1.2	5
			Tilting plane	2.0	
F_1	128	80-100	Open-field	1.2	6
			Tilting Plane	2.0	
F_2	101	110-125	Tilting plane	2.0	6
F_3	99	90-105	Rotarod	1.5	7
			Tilting plane	2.0	
F_4	115	100-115	Rotarod	1.5	7
			Tilting plane	2.0	
F_5 [2]	130	100-115	Rotarod	1.5	7
			Tilting plane	2.0	
F_6 [2]	103	60- 80	Rotarod	1.5	7
			Tilting plane	2.0	
F_7 [2]	112	60- 90	Rotarod	1.5	7
			Tilting plane	2.0	

[1] All intoxication tests were performed 30 minutes after ethanol injection except with the F_6 generation which was tested 45 minutes after ethanol injection.

[2] Control test values were obtained after saline injection.

Note: Blood alcohol determinations were made at the end of each session.

interval, a motor tilted the plane at a constant speed from horizontal to vertical. When the rat began sliding, the testing person stopped movement of the plane and recorded the sliding angle. The control values were taken 3 hours before the test. The rats were given three trials, and the mean of these was used as a control value. In the test situation with alcohol, the rats were also given three trials and the mean of these was used. The extent of intoxication was expressed as the decrease in mean sliding angle (in degrees) relative to control value. Reliability of the tilting plane test is indicated by the fact that the effect of ethanol on performance has been found to be proportional to the ethanol dosage (Arvola et al. 1958). In spite of the simplicity of the test, the performance on the tilting plane probably is a complicated function composed of vesticular and grasping reflexes, including a strong cortical component (Arvola et al. 1958). This method has also been used to measure acquired tolerance in rats (Malila 1978; Nikander and Pekkanen 1977).

Rotarod.

The rotarod test of motor coordination measures the ability of rats to run on a horizontal rotating rod without falling off. An accelerating rotarod was first described by Jones and Roberts (1968). The accelerating rotarod used in the present study was manufactured by Ugo Basile Company, Milan, Italy. It consists of a rotating rod approximately 6 centimeters in diameter, with a surface of finely corrugated plastic. The rod is divided into four compartments, 9 centimeters in width, by means of plastic disks 50 centimeters in diameter. Thus, four animals may be tested simultaneously, although in the present experiment each animal was tested individually. The rate of acceleration can be adjusted so that the time needed to reach the maximum speed (29 revolutions per minute) can be varied. A rate was used in the present study so that the apparatus reached its maximum speed at 165 seconds.

The number of seconds a subject stayed on the rod was measured automatically by a counter that was stopped when the animal fell from the rod and depressed a platform beneath it. The day before testing, the animals were familiarized with the apparatus by setting each rat on the rod in motion and allowing the rat to stay on the rod as long as possible. Three such trials were given. On the testing day, 3 hours before ethanol injection, three control trials without ethanol were performed on each rat and the mean of these was used as a control value.

In the test situation the rats were also given three trials, and the mean of these was used as the result. Each rat's performance was compared with its control score and the change in seconds used as the index of intoxication.

Open Field.

This test was only used in two first generations. The open-field apparatus employed in this study was described by K. Eriksson and Wallgren (1967). Ambulation, rearing, and time spent motionless in the open field were recorded in the 2-minute test sessions.

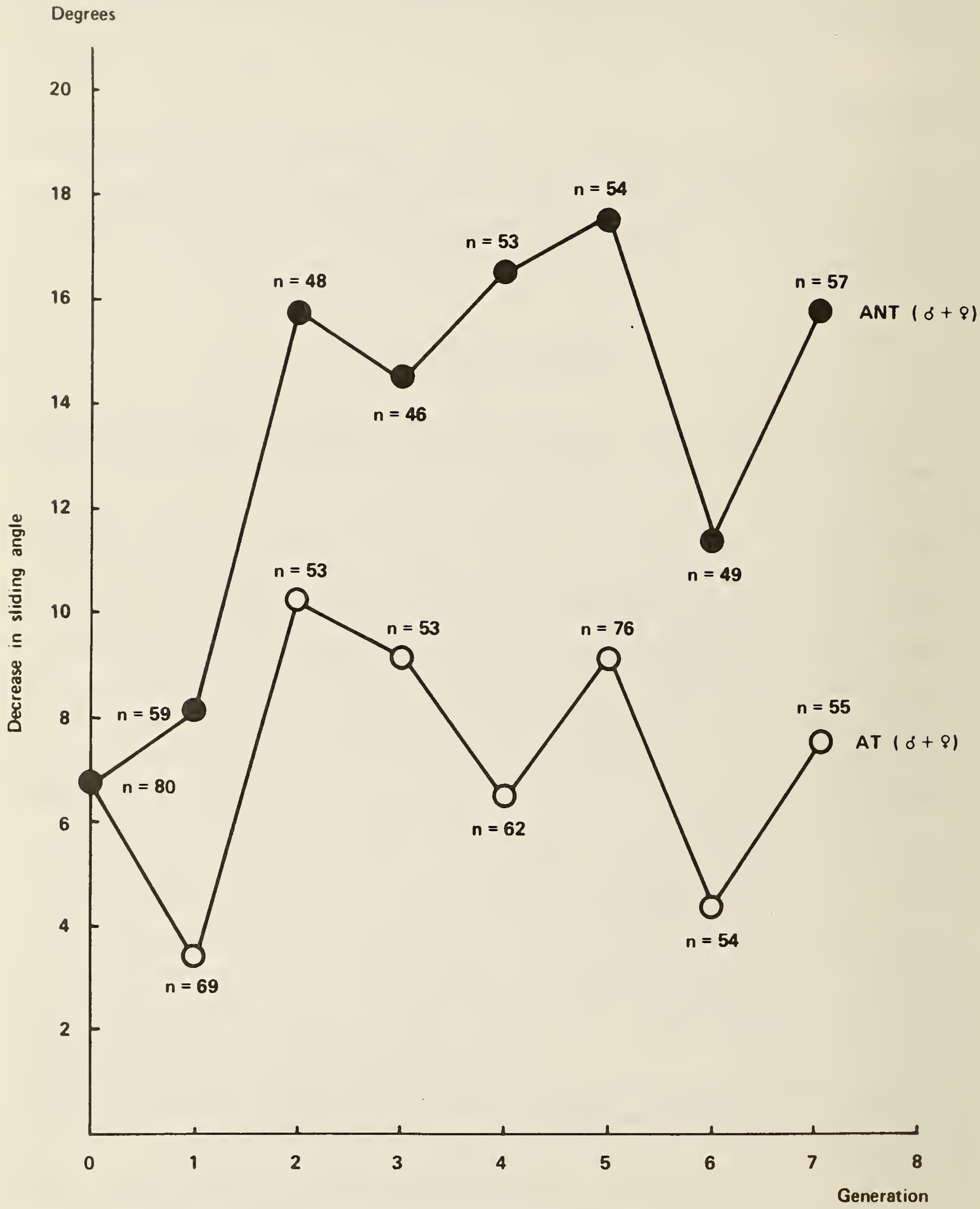
Selective Breeding

Seven offspring generations have now been tested with the method described above. In each generation there have been more rats than have been used for the intoxication tests (the numbers tested can be seen in figure 1 and in table 5). The animals for the intoxication tests were chosen randomly, however, so that they were from all the litters. No attention was paid to body weight or litter size. Those tested rats that had widely deviating blood alcohol concentrations were eliminated from the material. As mentioned before, the breeding animals have been selected primarily on the basis of their performance on the tilting plane, but the rotarod results were also considered. In every generation sib mating was avoided to eliminate restriction of individual variation. From the parental generation on, the rats showing the most debilitation from ethanol were mated to begin the alcohol nontolerant (ANT) strain, while the least affected were mated to begin the alcohol tolerant (AT) strain. Five breeding pairs for both strains were selected from the basic population, while six breeding pairs from the F_1 and F_2 generations were used. From the F_3 generation on, seven breeding pairs have been mated for both strains to produce the next generation. This means that about 20-30 percent of the tested animals in each generation were used for further breeding.

Methodological Points

From the very beginning of this study the intention has been to develop a statistical method for standardizing the phenotype so that all important factors, such as intoxication tests and blood alcohol

Figure 1. Separation Achieved by Genetic Selection for AT and ANT Strains



The effect of selective breeding in separating AT and ANT rat strains on the basis of the impairment of motor performances 30 minutes after ethanol (2 g/kg intraperitoneal) on the tilting plane test expressed as a decrease in the sliding angle (in degrees) relative to their own control values.

levels, could be reasonably combined. This problem has not yet been solved, so the selection of the breeding animals has been based on the raw score values. In this way we have been able to get an important selection effect during seven generations (see figure 1).

Material and Methods in the F_7 Generation

When it became evident that there was a significant strain difference on the tilting plane test between the AT and ANT rats in the seventh generation, we decided to examine in more detail the individual properties of that generation. Of the 159 rats in the F_7generation, 114 were used for intoxication tests. The general procedure for measuring intoxication was the same as described above, but additionally we wanted to get time-performance curves for both the tilting plane test and the rotarod test. So the ethanol tests were carried out at 30, 70, 110, and 150 minutes after ethanol injection. Determinations of blood alcohol concentrations were performed at the same points after the tests and blood acetaldehyde level was measured at the first two points after the dose of 2.0 g ethanol/kg body weight, using the gas chromatographic method (C. Eriksson 1973; C. Eriksson et al. 1977).

In this way we hoped to find out if there are differences in ethanol elimination rates, ethanol distribution, and ethanol diffusion rates that are important factors from the point of the goal of the selection. In the field of alcohol research, the role of acetaldehyde is an important question, and for that reason acetaldehyde was also measured.

Because recent findings suggest a difference in ethanol tolerance between the AA and ANA rat strains, with the strain preferring ethanol possessing a greater neural tolerance than the strain avoiding ethanol (Malila 1978; Nikander and Pekkanen 1977), it seemed important to note whether any differences in alcohol consumption occurred in AT and ANT strains. Therefore, we also studied the alcohol consumption of naive AT and ANT rats in three different periods. In this experiment 18 male and 9 female AT rats and 11 male and 7 female ANT rats (105 days of age) were used. A 10 percent (v/v) ethanol solution was offered by means of a two-bottle method (K. Eriksson 1968, 1969). The alcohol consumption was studied (1) when both ethanol and water were available (14 days), (2) subsequently when only ethanol solution was present (7 days), and (3) during a second free-choice period after forced consumption (14 days). Animals were housed individually with ad libitum Astra-Ewos®

standard food under a 12-hour light-dark cycle. The positions of the bottles were changed weekly during the free-choice periods. The quantities of water and ethanol were measured daily; body weight and food intake were measured twice a week.

Results of Genetic Selection for AT and ANT Strains

The separation achieved by the breeding program can be seen in figure 1, which presents the impairment of motor performance on the tilting plane 30 minutes after ethanol was given. Most of the difference between the strains was due to an impairment of performance in the ANT rats rather than to an improvement of performance in the AT rats. In the control values of the tilting plane and the rotarod, no significant differences were found between AT and ANT animals of any generation.

The open-field test was used in the two first generations. No consistent significant difference was found in the F_1generation between AT and ANT strains, and this method was not used in further generations.

Table 6 shows the body weights of both strains and sexes. As usual, the males are heavier in both strains, but there is no statistically significant difference in body weight between the AT and ANT strains. For the purpose of selection work, this means that possible differences found between strains in test performances or in consumption figures cannot be artifactually caused by differences in body weight.

Table 6. Mean Body Weights of the Alcohol Tolerant (AT) and Alcohol Nontolerant (ANT) Rats During Different Intoxication Tests (Mean ± S.D.)

Strain	Sex	*N*	Body Weight (g) First Test (Rotarod)	Body Weight (g) Second Test (Tilting Plane)
AT	M	29	274 ± 47	329 ± 46
ANT	M	29	276 ± 20	320 ± 18
AT	F	27	209 ± 30	241 ± 31[1]
ANT	F	28	208 ± 17	232 ± 20

[1] The second test for the AT strain was performed on an *N* of 26.

Table 7 shows blood ethanol levels 30, 70, 110, and 150 minutes after the acute i.p. ethanol injections of 2 g/kg used in the tilting plane test. There is no strain difference in blood alcohol level, which shows that diffusion rates played no role in the determination of phenotype. Nor is there any difference in the elimination rates between the AT and ANT strains. The elimination rate for females is a little higher than for males, as reported earlier in laboratory rats (K. Eriksson and Malmström 1967). Similar blood ethanol values and elimination figures were obtained after the smaller acute dose of ethanol (1.5 g/kg) used in the rotarod test.

Blood acetaldehyde (AcH) levels were measured from the same experimental material after the 2 g/kg ethanol dose (table 8). Blood AcH level seems to vary greatly between individuals, and the standard deviations are large. Nevertheless, there is no statistically significant difference between the AT and ANT rats in blood AcH levels after acute ethanol administration.

Table 8. Blood Acetaldehyde Levels 30 Minutes and 70 Minutes After Intraperitoneal Ethanol Injection (2g/kg) in the Alcohol Tolerant (AT) and the Alcohol Nontolerant (ANT) Rat Strains of the F_7 Generation (Mean ± S.D.)

			Acetaldehyde (nmol/ml)	
Strain	Sex	*N*	30 Minute	70 Minute
AT	M	29	13 ± 30	14 ± 20
AT	F	29	18 ± 18	12 ± 10
ANT	M	26	20 ± 17	19 ± 16
ANT	F	28	12 ± 13	12 ± 12

Note: None of the differences is statistically significant.

Table 7. Rate of Elimination of Ethanol After Intraperitoneal Injection of Ethanol (2g/kg) in the Alcohol Tolerant (AT) and the Alcohol Nontolerant (ANT) Rat Strains of the F_7 Generation (Mean ± S.D.)

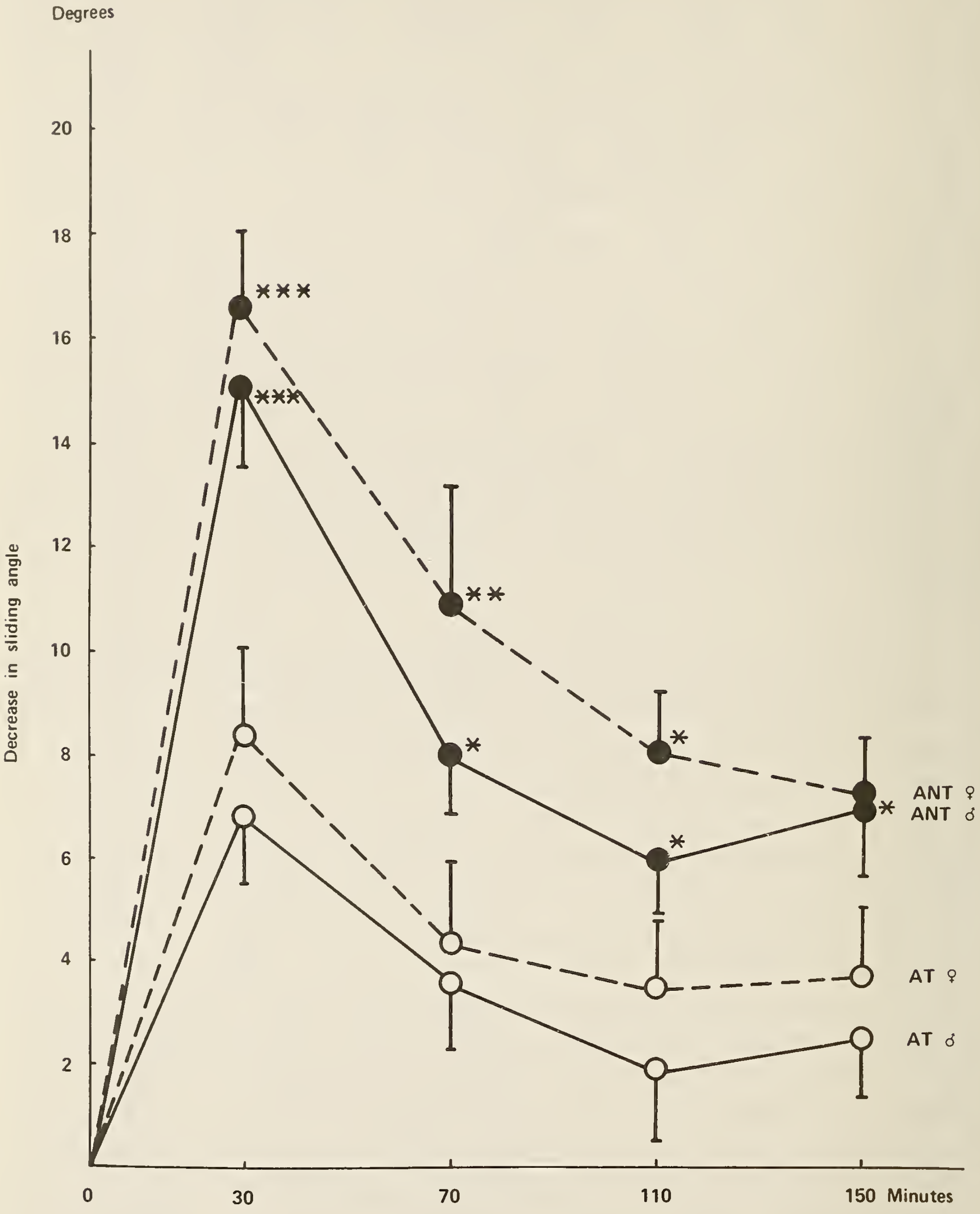
			Blood Ethanol Levels(μmol/ml)				Rate of Ethanol Elimination	Statistical Rate Comparison (probability)		
Strain	Sex	*N*	30 Minute	70 Minute	110 Minute	150 Minutes	(Mmole/kg/hr)	AT M	AT F	ANT M
AT	M	25	45.1 ± 6.7	43.1 ± 3.6	38.1 ± 4.1	31.9 ± 4.5	7.5 ± 1.5	–	–	–
AT	F	23	45.3 ± 5.5	40.8 ± 3.7	34.1 ± 2.5	25.8 ± 3.5	9.0 ± 1.1	<0.001	–	–
ANT	M	27	47.8 ± 5.5	43.8 ± 3.6	38.5 ± 3.0	32.6 ± 3.2	7.3 ± 1.1	N.S.	<0.001	–
ANT	F	27	48.5 ± 5.6	42.2 ± 3.8	34.8 ± 3.4	27.0 ± 3.5	8.6 ± 1.0	<0.005	N.S.	<0.001

Figure 2 shows the population means of the tilting plane test performances within the given time range after i.p. injection of 2 g/kg of ethanol. Individual frames show the differences between the sliding angle figures when compared with their own controls. Generally, the test performance seems to be a little better (the figures are smaller) in the males than the females, but the differences are not statistically significant. The strain difference in the tilting plane test is statistically significant, however, with the AT rats having a higher innate tolerance (smaller figures) over the given time period than the ANT rats. Strain differences are greatest at 30 minutes and 70 minutes after injection, during rising intoxication.

Figure 3 shows the respective results from the test performance on the rotarod after 1.5 g/kg i.p. ethanol injection. The results are in the same direction, with the AT strain having higher tolerance (smaller figures) than the ANT rats, but the strain differences are not statistically significant. Individual differences are great. Learning is strongly involved because the test performance values of the two last time points, 110 minutes and 150 minutes, are better (the figures are smaller) than the subjects' own controls before ethanol administration.

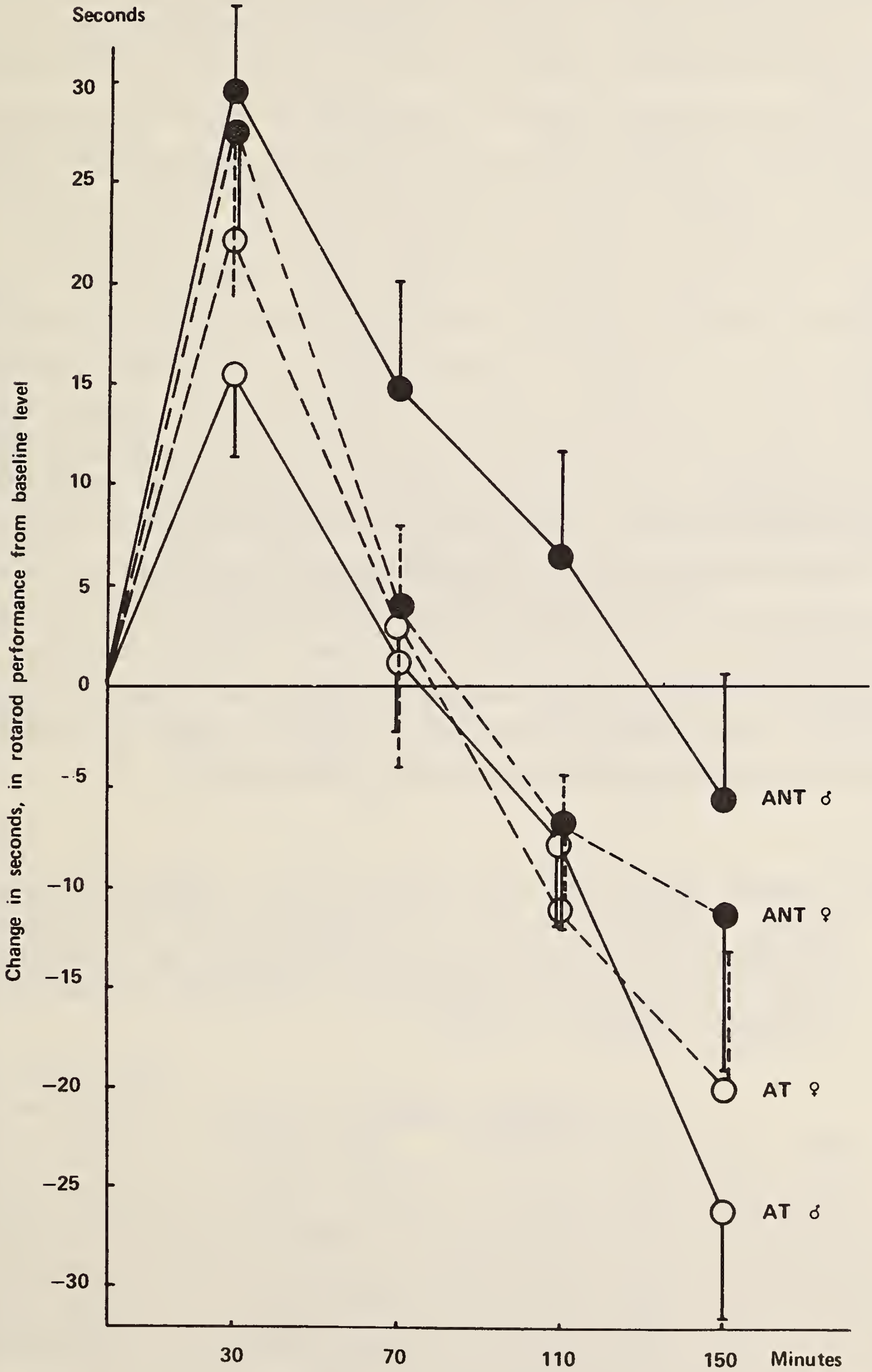
In addition to the selection program for innate ethanol tolerance, we wanted to test the voluntary ethanol intake of naive AT and ANT subjects. Table 9 shows the mean alcohol intake scores as population means during the initial 2-week ethanol free-choice period, the 1-week forced consumption period, and the 2-week free-choice period after forced consumption. There is a significant strain difference in voluntary ethanol intake during the initial free-choice period, with the AT strain drinking twice as much as ANT rats. There is, however, no difference during the forced ethanol consumption period and no significant difference during the free-choice period after forced consumption. Table 10 shows the daily total energy intake during the different alcohol consumption periods. There is a statistically significant difference between the strains in the daily total energy intake during the initial ethanol free-choice period. During forced ethanol consumption there is no significant difference between the strains, but during the last period, a significant strain difference can again be found for the females. Part of the difference in total energy intake can be attributed to the greater ethanol consumption of the AT rats, but the AT rats also tended to eat more food. There is, however, no significant difference in body weights between the AT and ANT rats during any of the alcohol consumption periods (table 11), and the changes in weight during the experiment were similar.

Figure 2. **Population Means of Tilting Plane Test Performance of Male and Female AT and ANT Rats**



The effect of 2.0 g/kg intraperitoneal ethanol injections on motor performance of the AT and ANT rat strains on the tilting plane, expressed as the decrease in sliding angle (in degrees) relative to their own control values in the F_7 generation. Each point in the figure represents the mean of the group. Vertical bars represent + or - SEM. Values were significantly different between the same sexes of the different strains (Student's *t*-test): $***p < 0.001$; $**p < 0.01$; $*p < 0.05$.

Figure 3. Population Means of Rotarod Test Performance of Male and Female AT and ANT Rats



The effect of 1.5 g/kg intraperitoneal ethanol injections on rotarod performance of the AT and ANT rats in the F_7 generation. Rotarod performance for each rate was compared with its baseline performance level determined prior to ethanol treatment. Each point in the figure represents the mean of the group. Vertical bars represent + or - SEM.

In addition to the selection program for innate ethanol tolerance, we wanted to test the voluntary ethanol intake of naive AT and ANT subjects. Table 9 shows the mean alcohol intake scores as population means during the initial 2-week ethanol free-choice period, the 1-week forced consumption period, and the 2-week free-choice period after forced consumption. There is a significant strain difference in voluntary ethanol intake during the initial free-choice period, with the AT strain drinking twice as much as ANT rats. There is, however, no difference during the forced ethanol consumption period and no significant difference during the free-choice period after forced consumption. Table 10 shows the daily total energy intake during the different alcohol consumption periods. There is a statistically significant difference between the strains in the daily total energy intake during the initial ethanol free-choice period. During forced ethanol consumption there is no significant difference between the strains, but during the last period, a significant strain difference can again be found for the females. Part of the difference in total energy intake can be attributed to the greater ethanol consumption of the AT rats, but the AT rats also tended to eat more food. There is, however, no significant difference in body weights between the AT and ANT rats during any of the alcohol consumption periods (table 11), and the changes in weight during the experiment were similar.

Table 9. Daily Intake of 10 Percent (v/v) Ethanol (g/kg Body Weight) in the F_7 Generation of the Alcohol Tolerant (AT) and Alcohol Nontolerant (ANT) Rat Strains During the Different Alcohol Consumption Periods (Mean ± S.D.)

Strain	Sex	*N*	Free Choice (ETOH vs. H_2O)	Forced Consumption (only ETOH)	Free-Choice after Forced Consumption (ETOH vs. H_2O)
AT	M	18	3.14 ± 1.49	5.57 ± 1.20	1.78 ± 1.02
			$p < 0.01$	N.S.	N.S.
ANT	M	11	1.78 ± 0.80	5.29 ± 0.71	1.29 ± 1.00
AT	F	9	2.70 ± 1.23	6.25 ± 0.72	2.22 ± 1.23
			$p < 0.05$	N.S.	N.S.
ANT	F	7	1.52 ± 0.56	6.23 ± 0.88	1.06 ± 0.87

Table 10. Total Energy Intake (kJ/kg Body Weight/Day in the F_7 Generation of the Alcohol Tolerant (AT) and Alcohol Nontolerant (ANT) Rat Stains During the Different Alcohol Consumption Periods (Mean ± S.D.)

Strain	Sex	*N*	Free Choice (ETOH vs. H_2O)	Forced Consumption only ETOH	Free Choice after Forced Consumption (ETOH vs. H_2O)
AT	M	18	803 ± 79	709 ± 104	719 ± 106
			$p < 0.025$	N.S.	N.S.
ANT	M	11	735 ± 55	659 ± 46	659 ± 100
AT	F	9	902 ± 73	807 ± 75	845 ± 46
			$p < 0.025$	N.S.	$p < 0.01$
ANT	F	7	805 ± 53	744 ± 62	761 ± 63

Table 11. Mean Body Weights (g) of F_7 Generation of the Alcohol Tolerant (AT) and Alcohol Nontolerant (ANT) Rats During the Different Alcohol Consumption Periods (Mean ± S.D.)

Strain	Sex	*N*	Free Choice (ETOH vs. H_2O)	Forced Consumption (only ETOH)	Free Choice after Forced Consumption (ETOH vs. H_2O)
AT	M	18	398 ± 68	409 ± 66	427 ± 69
			N.S.	N.S.	N.S.
ANT	M	11	417 ± 42	425 ± 46	446 ± 47
AT	F	9	278 ± 34	286 ± 26	294 ± 32
ANT	F	7	273 ± 38	277 ± 40	286 ± 42

Discussion

The voluntary ethanol intake of experimental animals in a two-bottle free-choice situation varies greatly between individuals, even in a well-controlled situation. During the course of this selection work, we could always find within the AA (high ethanol preference) strain some individuals that avoided the ethanol solution. In contrast, we could not find any heavily drinking individuals within the ANA population (K. Eriksson 1968, 1971, 1972). Nevertheless, the population means in all phenotypic measures of alcohol drinking behavior are clearly separate from each other, and there is practically no overlap between the AA and ANA populations. After the first clear appearance in the F_5 and F_6 generations, the strain difference has been constant.

Voluntary ethanol intake can easily be affected by some environmental factors. We know that the composition of the food influences the level of intake. This effect was clearly seen when we switched from our own Alko diet to the standard Astra-Ewos® diet, which was later found to contain an aldehyde dehydrogenase inhibitor: Both the AA and ANA strains had two times higher ethanol consumption while on the Alko diet than on Astra food (K. Eriksson and Närhi 1973). But even in this situation, the strain difference remained. Furthermore, we know that voluntary ethanol intake is greater in older animals than in younger ones, even when the effect of growth rate, with liquid requirement changes, is eliminated (K. Eriksson 1969). We had demonstrated earlier that after reaching maturity the ANA rats grow more rapidly than the AA rats (K. Eriksson 1969; K. Eriksson and Närhi 1973). Nevertheless, the strain difference in drinking behavior is clear at all ages tested, ranging from 30 days to nearly a year.

Different figures for body weight, total energy intake, and total fluid intake reached in different generations of this selection work show clearly that the phenotypic measure used for alcohol drinking behavior strongly affects the strains that develop during long-term selection. Probably the best phenotype for drinking behavior is some combination of all three measures (alcohol preference ratio, absolute ethanol intake per body weight, and alcohol energy preference ratio), which we eventually adopted. The simple linear combination of the three measures as such is not, however, likely to be the most satisfactory, because the basic traits involved in this genetic-environmental interaction (i.e., body weight, energy need, ability to use energy, liquid requirements, etc.) are not controlled by similar and quantitatively equal polygenic systems.

Optimally we would like to weight these phenotypic measures of alcohol drinking behavior in such a ratio that the resulting strains differ greatly in their ethanol intake, but have no difference in body weight, total energy intake, and fluid intake. If this goal could be reached, we would have more confidence that any other differences observed in the strains are actually related to the drinking behavior and are not solely the simple artifactual differences related to the selection method.

Much attention has been paid to the question of the alcohol concentration used in determining phenotype. Fuller (1964) studied the concentration preference in mice when six different concentrations of alcohol solution were offered simultaneously. He demonstrated that the inbred strains of mice show different concentration preferences. According to him, on the basis of these preference figures, an alcohol score can be calculated that can serve as a measure of the phenotype. Cicero and Myers (1968) presented experimental data showing that a single test solution can be employed as a measure of alcohol consumption if the concentration preference of different animals or strains is defined by using a concentration series starting with a 3 percent concentration and rising by 1 percent a day. A single test percentage is then selected that is 3 percentage points greater than the concentration just above the selection threshold, defined as the highest concentration at which the alcohol solution constitutes half of the animal's total fluid intake.

In the methodological part of this chapter, we discussed the weaknesses of preference ratio figures, especially in the course of genetic selection. These measurements are not much better if, instead of the two-bottle choice method, series of concentrations are used or more bottles are offered simultaneously. Drewek (1980) has recently done a large study in which he used an increasing concentration series of ethanol solution, in a three-bottle situation with one bottle empty. In his comprehensive genetic analysis, he obtained results showing that one concentration (10 percent) gives roughly the same results as six-eight concentrations. Lumeng et al. (1976) obtained similar results. The total amount of ethanol consumed by their alcohol-preferring (P) rats remained constant when the concentration of alcohol solution was varied between 10 percent and 30 percent. Earlier studies in our laboratory (K. Eriksson 1969) also showed that simultaneous presentation of five different ethanol concentrations gives practically the same individual differences when calculated as absolute ethanol intake per body weight. Therefore, from the point of genetic selection, the concentration used seems to be less important than other methodological factors.

The major purpose of the selection work for the AA and ANA rats has at all times been to raise two highly differing lines, one drinking much ethanol and the other avoiding it. This goal has been achieved. Additionally, we have tried to produce strains that are relatively free from artifactually related traits. How well we have succeeded in this will be seen in the future. For instance, the effects of the habituation period, during which 10 percent ethanol solution is the sole fluid, may have introduced artifacts. Perhaps the higher innate tolerance to ethanol, isopropanol, butanol, and barbital in AA rats is due to a genetic-environmental metabolic adaptation leading to higher neural tolerance in the course of the genetic selection. The greater potential for acquiring tolerance in the AA rats (Nikander and Pekkanen 1977) may also be a genetic-environmental interaction effect of the habituation period. During this period the animals are forced to drink large amounts of alcohol, which would have a greater effect on tolerance than the relatively small amounts consumed during free choice. Consequently, if the amount of tolerance acquired during the habituation period affected the quantity of alcohol subsequently chosen in the free-choice situation, animals that acquired more tolerance would have been selected for breeding in the AA line, and animals that acquired little tolerance would have been selected for the ANA line. It should be mentioned, however, that the AA rats drink more alcohol than the ANA rats even if they are started on free choice without a prior habituation period.

Another open question is the effect of time in the self-selection test period. Normally the free-choice test period in every generation has been 3 weeks, and the mean value of the last week has been used for determining the phenotype. We therefore have selected animals that differ specifically in voluntary consumption at this time in the course of their ethanol experience, and it is possible that different results would have been obtained if drinking at some other time had been used for the phenotype. There are, however, several reasons for thinking that the measure used is a more reliable and relevant determinant of phenotype than drinking during the first few days of free-choice access to alcohol. Typically rats drink relatively little alcohol at first and then show a progressive increase, approaching an asymptotic level after 3-4 weeks and showing little change thereafter, even if tested for several months. In addition to being lower, the alcohol intake during the first days is less stable within individuals across time than the consumption after several weeks of access, perhaps because it is more affected by neophobia. The drinking levels after several weeks of experience, on the other hand, are more likely to be affected by learning related to positive or negative reinforcement from pharmacological effects of alcohol. Finally, whatever relevance our rat studies have to human drinking would relate to consumption after relatively long-term prior experience, not to behavior on the first exposure to alcohol.

The original purpose of the outbreeding selection method used was to try to maintain the heterogeneity of these strains. In that case one can assume that differences found between the strains are more likely to be truly correlated with drinking behavior. The enzyme polymorphism study done with these animals (K. Eriksson et al. 1976) suggests, however, that outbreeding techniques are not very effective in maintaining heterozygosity in laboratory rats. It is true, of course, that inbreeding is unavoidable if an outbred laboratory stock is maintained at a moderate population size. The low number of polymorphic loci in the mixed strain, the basic population of AT and ANT rats, indicates that the three laboratory strains most commonly used are relatively similar. Nevertheless, the clear differences in many physical traits of the AA, ANA, AT, ANT, and mixed rats undoubtedly reflect rather wide genetic variety. This variety is also shown by the magnitude and rapidity of the selection responses of the AA and ANA rats when different phenotypic measures are used as a tool for genetic selection.

Recently, two other rat strains comparable with the AA and ANA rats have been developed by selective inbreeding for their different ethanol preferences (Li and Lumeng 1976; Lumeng et al. 1976). The general procedure of this study is quite similar to that used in developing the AA and ANA rats. Some methodological differences, however, can be found: The habituation period consisted of only 4 days in this study, and the rats were younger (at the onset of puberty) when the self-selection was started. These differences appear not to have affected the final goal of the selection, because the drinking scores of alcohol-preferring (P and AA) and alcohol-nonpreferring (NP and ANA) rats are quite comparable with each other. Unlike the AA and ANA strains, however, the P and NP strains were not found to differ significantly in their acetaldehyde metabolism. They also did not differ with regard to ethanol metabolism. No data are available yet concerning their tolerance to ethanol.

The naive offspring subjects of our AA and ANA strains do seem to have inherited differences in acute tolerance or rapid tolerance to ethanol. This finding led us to try a new selection procedure for AT and ANT rats. In the well-controlled test situation the estimation of phenotype seems to be more easily handled and stabilized when studying acute tolerance than when studying voluntary drinking behavior. The most likely artifactual traits related to the test situation would be individual differences in the diffusion rate and elimination rate of ethanol. The goal of the selection was to produce animal strains differing in intoxication after the same acute injection of ethanol with equal blood ethanol levels. The results clearly show that the strain differences in acute intoxication and tolerance found with two testing methods cannot be explained by different body weights. The identical ethanol elimination curves during 150 minutes after injection indicate that the diffusion and elimination of ethanol happen similarly in both strains. So the goal has been achieved. The selection response seems to be greater in ANT animals because their motor impairment in every generation has gotten progressively worse while the performance of AT animals has not improved much from the original base level.

Another important result is that the control mean values after saline injection in both test methods, tilting plane and rotarod, are very similar in both strains. This shows that we have not developed strains differing merely in their capacity to perform on the tests and mistaken that for a difference in acute intoxication. Striking innate differences in motor activity would probably also have been reflected as differences in these baseline values.

The small test of the voluntary ethanol intake of naive AT and ANT rats showed a strain difference, with the AT strain having higher ethanol consumption. There was no difference between the strains in ethanol intake during the habituation period when only ethanol was available, perhaps reflecting the similar capacity of the strains to metabolize alcohol. We cannot yet conclude that higher voluntary ethanol preference is inherited along with higher innate tolerance to acute ethanol intoxication, but these initial results with AT and ANT rats do at least suggest that this may be possible. Genetically this is also easy to accept because the AA and ANA subjects are crossbred into the basic population of AT and ANT rats.

Differential voluntary ethanol consumption does not always follow different levels of tolerance to ethanol, as is demonstrated by the special rat strains developed by Riley et al. (1976). They used the degree of motor impairment as measured in a stabilimeter following a 1.5 g/kg dose of ethanol as their phenotypic measure for selectively breeding a “most affected” (MA) and a “least affected” (LA) strain. The MA animals now generally show a 90 percent reduction in spontaneous motor activity following the alcohol challenge, while the LA strain shows only a 40 percent reduction (Riley and Lochry 1977; Worsham, Freed, Lochry, and Riley 1977). Unlike our AT and ANT strains, the MA and LA strains do not differ in their voluntary ethanol consumption (Worsham, Riley, Ananham, Lister, Freed, and Lester 1977). Otherwise, the results obtained by Riley et al. (1978) parallel those we have gotten. The difference in tolerance between the LA and MA strains has developed primarily from a decrease in the MA strain, while the LA strain has tended to show a relatively constant tolerance across generations (Riley et al. 1978), just as the difference between the AT and ANT strains has developed primarily from a progressively lower tolerance across generations in the ANT strain. Also like the AT and ANT strains, there are no differences between the LA and MA strains in blood alcohol concentrations following ethanol administration (Riley et al. 1977), and differential absorption of alcohol also fails to account for the differences in tolerance (Worsham, Freed, Lochry, and Riley 1977). The LA strain also shows more acquired tolerance than the MA strain, developing more tolerance following regular alcohol exposure through intubation on both motor activity and sleeping time measures (Riley and Lochry 1977). The spontaneous activity of the LA strain is also less affected by phenobarbital than that of the MA strain (Riley et al. 1978). In addition to voluntary ethanol consumption, the only results differentiating these strains from our AT and ANT strains are that the LA rats have slightly heavier body weights than MA animals and greater activity scores in running wheels (Riley et al. 1977; Worsham, Freed, Lochry, and Riley 1977; Worsham, Riley, Ananham, Lister, Freed, and Lester 1977).

It is difficult to find a logical explanation for the higher energy intake of AT rats compared with ANT rats. This difference is very clear and in the same direction throughout the whole test period of 5 weeks, including both free-choice periods and the habituation period. Because body weight is the same for both strains, the differences in energy intake between AT and ANT rats cannot be explained trivially as due to the differences in fat metabolism, basic metabolism, weight-apparatus interactions, etc.

It is naive to believe that a single animal model, or even specially selected lines as such, could explain human alcoholism. One can conclude solely on the basis of methodological reasons that the generalization of the results reached with special strains is difficult. When trying to cover the metabolism and behavior of such a complex behavior as human alcoholism, we need many different animal models and specially selected lines. In our laboratory, we have selected high and low lines for voluntary ethanol drinking behavior and acute ethanol intoxication or tolerance. The results suggest that these two ethanol-related behaviors may be genetically linked in some way. Trying to determine how they are joined is the next step.

References

Arvola, A.; Sammalisto, L.; and Wallgren, H. A test for level of alcohol intoxication in the rat. *Q J Stud Alcohol*, 19:563-572, 1958.

Brewster, D.J. Ethanol preference in strains of rats selectively bred for behavioral characteristics. *J Genet Psychol*, 115:217-227, 1969.

Cicero, T.J., and Myers, R.D. Selection of a single ethanol test solution in free-choice studies with animals. *Q J Stud Alcohol*, 29:446-448, 1968.

Curley, J.J., and Pelas, A. Comparative studies of litters obtained from Long-Evans and Sprague-Dawley rat stocks. *Lab Anim Care*, 19:716-719, 1969.

Drewek, K.J. Inherited drinking and its behavioral correlates. In: Eriksson, K.; Sinclair, J.D.; and Kiianmaa, K., eds. *Animal Models in Alcohol Research.* London: Academic Press, 1980. pp. 35-49.

Eriksson, C.J.P. Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochem Pharmacol*, 22:2283-2292, 1973.

Eriksson, C.J.P.; Sippel, H.W.; and Forsander, O.A. The determination of acetaldehyde in biological samples by head-space gas chromatography. *Anal Biochem*, 80:116-124, 1977.

Eriksson, K. Genetic selection for voluntary alcohol consumption in the albino rat. *Science*, 159:739-741, 1968.

Eriksson, K. Factors affecting voluntary alcohol consumption in the albino rat. *Ann Zool Fenn*, 6:227-265, 1969.

Eriksson, K. Rat strains specially selected for their voluntary alcohol consumption. *Ann Med Exp Biol Fenn*, 49:68-72, 1971.

Eriksson, K. Behavioral and physiological differences among rat strains specially selected for their alcohol consumption. *Ann NY Acad Sci*, 197:32-41, 1972.

Eriksson, K., and Malmström, K.K. Sex differences in consumption and elimination of alcohol in albino rat. *Ann Med Exp Biol Fenn*, 45:389-392, 1967.

Eriksson, K., and Närhi, M. Specially selected rat strains as a model of alcoholism. In: Spiegel, A., ed. *The Laboratory Animal in Drug Testing.* Stuttgart: Springer Verlag, 1973. pp. 282-301.

Eriksson, K., and Wallgren, H. Behavior of rats under the influence of ethyl alcohol in an open-field situation. *Scand J Psychol*, 8:257-267, 1967.

Eriksson, K.; Halkka, O.; Lokki, J.; and Saura, A. Enzyme polymorphism in feral, outbred and inbred rats (*Rattus norvegicus*). *Heredity*, 37:341-349, 1976.

Fuller, J.L. Measurement of alcohol preference in genetic experiments. *J Comp Physiol Psychol*, 57:85-88, 1964.

Jones, B.J., and Roberts, D.J. The quantitative measurement of motor inco-ordination in naive mice using an accelerating rotarod. *J Pharm Pharmacol*, 20:302-304, 1968.

Li, T.-K., and Lumeng, L. Alcohol metabolism of inbred strains of rats with alcohol preference and nonpreference. (Abstr. 42). In: Thurman, R.G.; Williamson, J.R.; Drott, H.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems.* Vol. III. New York: Academic Press, 1977.

Lumeng, L.; Hawkins, T.D.; and Li, T.-K. New strains of rats with alcohol preference and nonpreference. (Abstr. 38). In: Thurman, R.G.; Williamson, J.R.; Drott, H.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems.* Vol. III. New York: Academic Press, 1977.

Malila, A. Intoxication effects of three aliphatic alcohols and barbital on two rat strains genetically selected for their ethanol intake. *Pharm Biochem Behav*, 8:197-201, 1978.

Nikander, P., and Pekkanen, L. An inborn alcohol tolerance in alcohol-preferring rats. The lack of relationship between tolerance to ethanol and the brain microsomal (Na^+K^+) ATPase activity. *Psychopharmacology*, 51:219-223, 1977.

Riley, E.P., and Lochry, E.A. Effects of initial tolerance on acquired tolerance to alcohol in two selectively bred rat strains. *J Drug Alcohol Depend*, 2:485-494, 1977.

Riley, E.P.; Freed, E.X.; and Lester, D. Selective breeding of rats for differences in reactivity to alcohol: An approach to an animal model of alcoholism. I. General procedures. *J Stud Alcohol*, 37:1535-1547, 1976.

Riley, E.P.; Lochry, E.A.; and Freed, E.X. Differential tolerance to pentobarbital in rats bred for difference in alcohol sensitivity. *Psychopharmacology*, 58:167-170, 1978.

Riley, E.P.; Worsham, E.D.; Lester, D.; and Freed, E.X. Selective breeding of rats for differences in reactivity to alcohol: An approach to an animal model of alcoholism. II. Behavioral measures. *J Stud Alcohol*, 38:1705-1717, 1977.

Rusi, M.; Eriksson, K.; and Mäki, J. Genetic differences in the susceptibility to acute ethanol intoxication in selected rat strains. In: Gross, M.M., ed. *Alcohol Intoxication and Withdrawal*. Vol. IIIA. (*Vol. 85a of Advances in Experimental Medicine and Biology*.) New York: Plenum Press, 1977. pp. 97-109.

Worsham, E.D.; Freed, E.X.; Lochry, E.A.; and Riley, E.P. Generality of differential sensitivity to alcohol in selectively bred rats. *Physiol Psychol*, 5:429-432, 1977.

Worsham, E.D.; Riley, E.P.; Anandam, N.; Lister, P.; Freed, E.X.; and Lester, D. Selective breeding of rats for differences in reactivity to alcohol: An approach to an animal model of alcoholism. III. Some physical and behavioral measures. In: Gross, M.M., ed. *Alcohol Intoxication and Withdrawal*. Vol. IIIA. (*Vol. 85a of Advances in Experimental Medicine and Biology*.) New York: Plenum Press, 1977. pp. 71-81.

Finnish Selection Studies on Alcohol-Related Behaviors: Factors Regulating Voluntary Alcohol Consumption

C.J. Peter Eriksson*

The aim of alcohol research with experimental animals is to find answers with relevance to human alcoholism. It is well accepted that most biochemical, physiological, and pharmacological actions of alcohol are comparable in humans and experimental animals at least qualitatively. The same is also believed to be true for some alcohol-related behaviors such as alcohol intoxication and physical dependence measured as intensity of withdrawal. When the relationship between humans and experimental animals with regard to voluntary alcohol consumption is considered, the opinions are more divergent. This is understandable because of the general knowledge of environmental factors involved in the etiology of human alcoholism. However, it has become increasingly evident that there is an inherited genotype that, together with environmental factors, forms the alcoholic phenotype. (For a review of this topic see K. Eriksson 1975.) The recognition of genetic factors involved in human alcohol-drinking behavior provides a reason for the investigation of corresponding inherited mechanisms in experimental animals.

Selective outbreeding provides an excellent method for investigating genetic mechanisms involved in the regulation of alcohol-related behaviors. The advantages over that of comparing different inbred strains, which do or do not exhibit the alcohol behavior in question, have been described previously (K. Eriksson 1975). Thus, any metabolic, physiological, or pharmacological differences found between the AA (Alko, alcohol) and the ANA (Alko, nonalcohol) outbred rat strains (developed in the Research Laboratories of the State Alcohol Monopoly, Finland) should be related to the alcohol-drinking behavior of the animals. The objective of this chapter is to survey the strain differences and to use the differences for the development of models for the regulation of voluntary alcohol consumption.

---

*The author wishes to thank his colleagues, Liisa Ahtee, Kalervo Eriksson, Kalervo Kiianmaa, Jaakko Linkola, Kai O. Lindros, Leena Pekkanen, and J. David Sinclair, for valuable criticism of the manuscript. He also thanks Rodney Baker and Richard A. Deitrich for helpful discussions.

The AA and ANA Strain Differences and the Regulation of Voluntary Alcohol Consumption: General Aspects

Before the AA and ANA strain differences can be analyzed, some basic concepts must be clarified. First and perhaps most important is the fact that the regulation of alcohol-drinking behavior in experimental animals or humans has to involve two classes of regulatory factors. The positive (+) simply refers to factors that increase alcohol consumption and the negative (-) to factors that decrease alcohol consumption. Thus, the drinking behavior in a given situation is regulated by the combination of different + and - factors. This somewhat trivial basic concept is stated because the implications have often been neglected. One example is the comparison of inbred strains. Inbred animals have a particular constellation of genes that were fixed early in their breeding history. Comparison of two inbred strains for a variety of effects will result in a number of correlations that may be entirely fortuitous; the correlation of alcohol preference with any given factor in inbred strains may not be meaningful insofar as cause and effect relationships are concerned. Even if the inbred strains were selected for high or low ethanol preference, the factor responsible for the differences may be relatively minor and much more powerful factors may have been lost in the inbreeding process. Thus, conclusions obtained from inbred strain comparisons must be regarded as tentative, at least until appropriate methods, such as correlation analysis with an F_2 crossbred population, have been applied.

Another important consideration is the fact that it is the combination of different factors that regulates alcohol-drinking behavior.

This point is especially applicable to those situations in which animals are tested in a way that alters their drinking habits. In most of these cases the speculations focus on one factor without sufficient consideration of other regulatory factors that have been changed also. Perhaps one reason for this has been the lack of agreement on the basic factors involved in the regulation of drinking.

Another concept regarding the analysis of drinking behavior involves the different measures used in these types of studies. The use of these different measures—the amount of ethanol solution consumed as a percentage of the total fluid consumed (E/T), the amount of alcohol per unit of body weight, and the percentage of the total energy intake obtained from alcohol—was discussed previously (K. Eriksson 1975). In the outbred selection of the AA and ANA strains the amount of alcohol per unit of body weight has been used. The E/T ratio was used in the beginning of the selection (K. Eriksson 1969); at a later stage of the selection, the caloric preference score was used (see chapter by K. Eriksson and Rusi, in this report). Even if these three measures correlate significantly with each other (K. Eriksson 1969), the relative importance of the different factors regulating voluntary alcohol consumption may have changed subsequent to the changes in the selected traits. This possibility will be exemplified later.

A final concept that should be pointed out is the design of all the AA and ANA preference tests that have included the "habituation period" during which 10 percent (v/v) ethanol has been the sole fluid for 10 days (K. Eriksson 1968). Thus, all the aversive or reinforcing mechanisms that will be described later in the text may have been strengthened as a result of the conditioned aversion or reinforcement derived from the habituation period.

Table 1 lists all the metabolic, physiological, pharmacological, and behavioral differences reported so far from the AA and ANA strains. In principle, all these differences should be related either directly or indirectly to the differences in the drinking behavior. Based upon the concept described above, these differences will be analyzed in the following sections.

Table 1. Differences Found Between the Ethanol-Avoiding (ANA = ALKO, Nonalcohol) and Ethanol-Preferring (AA = ALKO, Alcohol) Animals

Parameter	Difference	Generation	Reference
Body weight	ANA > AA	F_8	Eriksson, K. (1968)
Brain serontonin, Rectal temperature	AA > ANA	F_{17}	Ahtee and Eriksson (1972)
Open-field activity	ANA > AA	F_{17}	Eriksson, K. (1972)
Blood and liver acetaldehyde	ANA > AA[1]		
Ethanol oxidation rate			
Mitochondrial redox state	AA > ANA[1]	F_{17}	Eriksson, C.J.P. (1973)
Blood ketone bodies	ANA > AA	F_{17}	Forsander and Eriksson, C.J.P. (1972)
Recovery from inhibition of K^+ transport in brain	AA > ANA[1]	F_{18}	Boguslawsky and Nikander (1972)
Food utilization	ANA > AA	F_{19}	Eriksson, K. and Närhi (1973)
Sleeping time	ANA > AA[1]	F_{20}	Rusi et al. (1977)
Brain dopamine	AA > ANA		
Brain weight	ANA > AA	F_{22}	Ahtee and Eriksson (1975)
Intracardiac conduction, Duration of tachycardia	AA > ANA[1]	F_{22}	Hillbom and Boguslowsky (1978)
Liver ADH	ANA > AA		
Liver ALDH	AA > ANA	F_{23}	Koivula et al. (1975)
Tilting plane performance	AA > ANA[1]		
Brain Mg^+ (Na^+ K^+) ATP ase	AA > ANA	F_{23}	Nikander and Pekkanen (1977)

Table 1. Differences Found Between the Ethanol-Avoiding (ANA = ALKO, Nonalcohol) and Ethanol-Preferring (AA = ALKO, Alcohol) Animals

Parameter	Difference	Generation	Reference
Urine outputs (urine, Na^+ and K^+) after $H_2 0$ load	AA > ANA[1]	F_{24}	Linkola (1976)
Alcohol and barbital-induced intoxication	ANA > AA	F_{26}	Malila (1978)
Urine outputs (urine, Na^+ and K^+)	ANA > AA[1]		
Urinary vasopressin	AA > ANA[1]	F_{28}	Linkola et al. (1977)
Body weight	AA > ANA	F_{29}	Eriksson, K., and Rusi (in this book)
Urinary vasopressin after H_2O load	ANA > AA[1]		
Urinary C-AMP after H_20 load	AA > ANA[1]	F_{29}	Linkola and Fyhrquist (1978)
Alcohol deprivation effect	AA > ANA	F_{30}	Sinclair (1979)

[1] In the presence of ethanol.

Relationship Between Alcohol Consumption and the Energy Requirement

A body weight difference, with both males and females of the ANA strain being heavier than the AA rats, was originally reported from the F_8 generation (K. Eriksson 1968, 1969). At this point the body weight difference was thought to relate to inherited metabolic differences that could be associated with the regulation of alcohol consumption. The body weight difference was still a prominent feature in subsequent generations as reported from F_{16} (K. Eriksson 1971, 1972). The physiological explanation of the body weight differences was found in the F_{19} generation when K. Eriksson and Nähri (1973) discovered that the food utilization efficiency index (food consumed/gain in weight × mean body weight) was significantly higher in the males and females of the AA strain than in those of the ANA strain with two different diets. In other words, the naive rat's (no experience with alcohol) energy requirement (caloric need) was higher in the AA strain. Moreover, the food intake (per unit body weight) of the female animals of both strains with both diets was found to be significantly higher than that of the corresponding males. This discovery provided an explanation for the sex differences in body weight as found in the beginning of the selection (K. Eriksson 1968, 1969). After F_{19} the selection was continued primarily on the basis of caloric preference, and from F_{29} on, the basis for the selection became a combination of the E/T, ethanol consumption per unit of body weight, and the caloric preference (K. Eriksson and Rusi chapter in this report). The body weight difference started to change in the opposite direction, with the AA rats being significantly heavier than the ANA strain in generation F_{29} (table 1 from K. Eriksson and Rusi, in this report). The pattern of these body weight differences led to the hypothesis that the former differences, with the ANA strain being heavier than the AA strain, merely represented an artifact caused by a selection pressure on the body weight without relevance to alcohol drinking (K. Eriksson 1975; K. Eriksson and Rusi, in this report).

Another possible explanation of the observed inherited body weight difference is associated with the regulation of voluntary alcohol intake. Thus, ethanol would serve as a reinforcing resource of calories, and the smaller animals (with higher caloric need) would consume more alcohol per body weight. This would explain not only the earlier strain differences regarding the body weight but also why the females (with lower body weight and higher energy requirement) of both strains consumed more and had a higher ethanol preference, as observed in generations 8-20 with the Alko diet (K. Eriksson 1968, 1969, 1971, 1972; K. Eriksson and Närhi 1973). The following explanation is suggested for the subsequent development of the opposite body weight differences, with the AA rats becoming heavier than the ANA rats. In the ANA animals, which have lower body weight (and higher caloric need), the calories would be taken preferably from food rather than ethanol because of the dominance of the aversive factors for alcohol in this strain. Thus, the ratio of ethanol calories to total calories would decrease, meaning that the ANA selection would be associated with a selection pressure for low body weights. However, in the AA animals this would not be likely to occur because the aversive effect of ethanol is small (or absent). Higher caloric need could in this case increase the ethanol calories as much as the food calories, and no selection pressure on body weight would occur as it does in the ANA line.

If the body weight/energy requirement/ethanol intake relationship is true for the strains as well as for the sexes, two logical consequences should follow the change of the selection in favor of caloric preference. Overall ethanol consumption and sex differences regarding ethanol consumption should both decrease. Indeed, ethanol consumption did decrease drastically and the former sex differences disappeared when the selection was changed in favor of the caloric preference. Unfortunately, the diet was changed at the same time, and the new Astra diet (from Astra-Ewos Ab, Södertälje, Sweden) was found later to contain an aldehyde dehydrogenase inhibitor, which probably was the main cause of the reduction in the ethanol consumption (this aspect is discussed later). Thus, no conclusions regarding the body weight/caloric need/alcohol consumption relationship can be drawn for this time period. Later the inhibitory factor in the Astra diet was eliminated, and the selection parameter changed to the combination of E/T, alcohol consumption per unit body weight, and caloric preference. However, even in this case the alcohol consumption in the AA strain is still lower than it was, and the former sex differences have disappeared (table 3 from Eriksson and Rusi, in this report), thereby supporting the importance of the energy requirement in the regulation of voluntary alcohol consumption. These relationships are an excellent example of how important the choice of the selection parameters is.

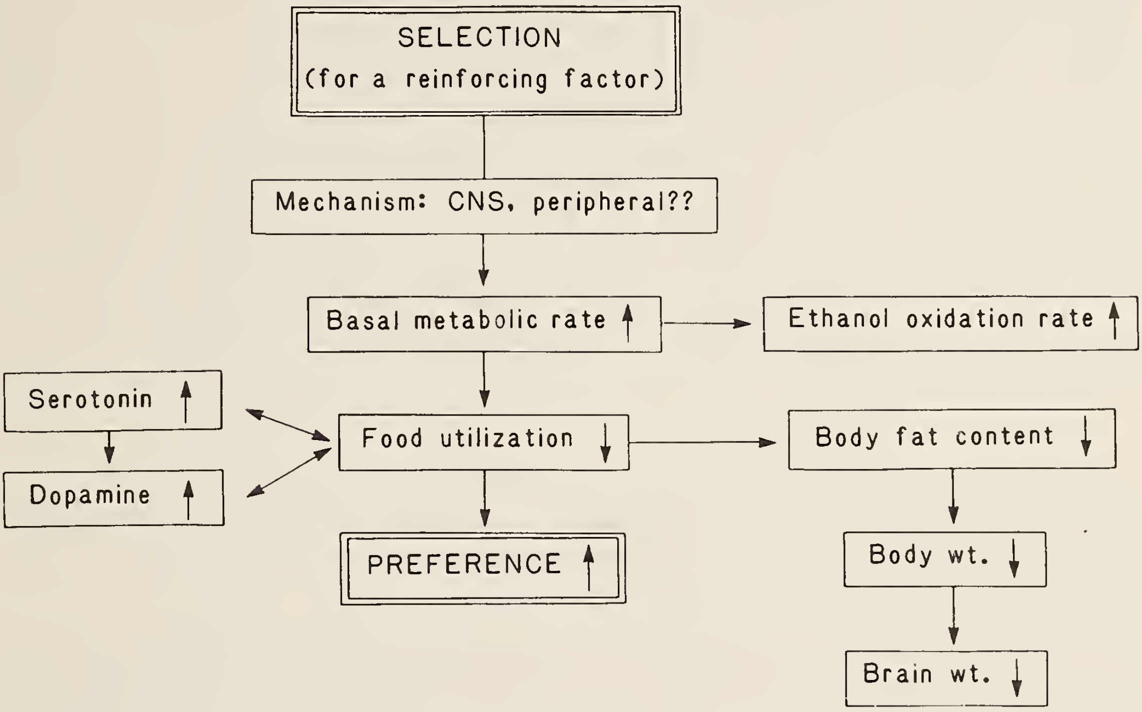
A relationship between the "caloric need" and alcohol consumption would not be a very surprising selection result per se. This relationship is suggested by a substantial amount of data (Forsander and K.

Eriksson 1972; Wallgren and Barry 1970). As demonstrated by the same references, there are also a number of studies questioning the relationship. However, most of the conclusions regarding the relationship between caloric need and alcohol intake are hampered by the fact that they have been drawn from studies where animals have been under some kind of treatment, which may or may not have changed other possible regulators of alcohol consumption. In fact, it appears that there is no study where the relationship, or its absence, has been comprehensively studied during control conditions (without environmental changes). However, from the AA and ANA studies it seems that the relationship does exist. It is therefore suggested that every study concerning changes in alcohol consumption should be controlled for possible correlated changes in the food intake before other conclusions regarding the regulation of the alcohol consumption are drawn.

A number of the differences demonstrated in table 1 may be connected to the food utilization differences between these strains. A possible model for these interrelations is shown in figure 1. A higher body fat content in the ANA is suggested to be the link between the higher body weight and food utilization. The indication of this link could be the higher blood ketone body concentrations found after an oral n-butyrate load in the ANA strain (Forsander and C. Eriksson 1972). This relation could also explain earlier data by Forsander and Salaspuro (1962), who found a negative correlation between ketone body excretion (after oral fat administration) and alcohol preference.

A more active basal metabolism is suggested to cause the higher food intake and energy requirement in the AA animals. The higher metabolic activity is also supported by the finding of an elevated rectal temperature in the AA rats (Ahtee and Eriksson 1972). Moreover, the faster basal metabolic rates would explain the ethanol oxidation rate differences obtained between the female AA and ANA rats reported by C. Eriksson (1973). This would also explain the mechanism for the sex differences obtained in both strains, with the females oxidizing ethanol significantly faster than the males (C. Eriksson 1973) which previously had been observed in rats of Wistar origin (K. Eriksson and Malmström 1967). It is possible that there has been a selection pressure in the AA line for higher metabolic rates, because the earlier AA generations drank alcohol amounts close to their physiological limit (K. Eriksson 1971). This idea of a "metabolic tolerance" was originally suggested by Forsander and Salaspuro (1962). However, the "metabolic tolerance" may at most provide a contributory explanation for the development of the food utilization

Figure 1. A Model for Interrelations Among Food Utilization, Metabolism, and Ethanol Preference



and body weight differences between the AA and ANA strains, because different ethanol oxidation rates were found only between the female rats of the two strains.

The higher brain serotonin concentration and the elevation of this transmitter after chronic ethanol treatment found by Ahtee and Eriksson (1972, 1973) in the AA animals were proposed as a possible mechanism for a reinforcement of ethanol intake. However, in a recent investigation by Kiianmaa (1975), who used three different methods of lowering the brain serotonin levels in AA rats, it was concluded that serotonin does not play any important role in the regulation of alcohol consumption. Furthermore, the literature is full of contradictory reports of serotonergic effects on alcohol preference (see the review by Collins 1979). A partial explanation of these contradictions would be that the strain differences in serotonin levels would not be related to a specific ethanol mechanism but rather to the food utilization differences of the strains (figure 1).

Support for a relationship between brain serotonin and food intake differences is provided by the positive correlation between total brain serotonin level and its precursor, the plasma tryptophan level, found by Fernström and Wurtman (1972). Thus, the relatively nonspecific brain serotonin elevation found in the AA strain (Ahtee and Eriksson

1973) could well have been the result of an elevation in the plasma tryptophan level in the AA rats, caused by the higher food intake and lower food utilization in these animals. The effects of p-chlorophenylalanine and fenfluoramine, decreasing brain serotonin levels and alcohol consumption (Myers and Veale 1968; Opitz 1969), also could be explained partly by the anorexic effects of these drugs found in the same studies. The higher brain dopamine concentration found in the AA rats compared with the ANA strain (Ahtee and Eriksson 1975) could simply reflect similar mechanisms, a compensation effect for the elevated serotonin levels, or dopamine could be involved in the regulation of the food intake, as indicated by the findings of Stricker and Zigmond (1974).

Relationship Between Alcohol Consumption and Acetaldehyde Metabolism

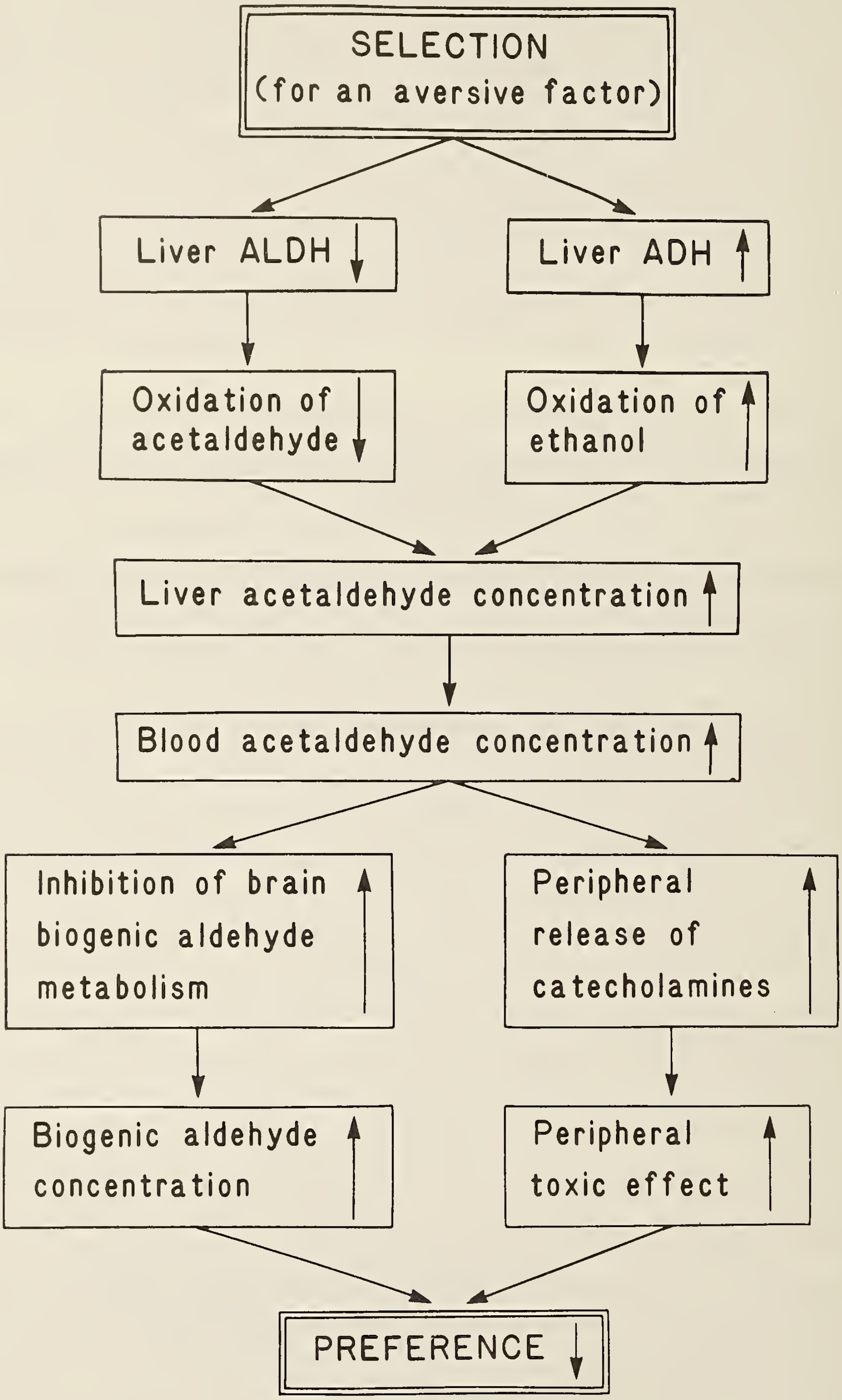
In the first strain comparison in which the ethanol metabolism was investigated in vivo and in vitro in isolated perfused liver, substantial differences were found in the bood and liver acetaldehyde concentrations, with both sexes of the AA strain displaying lower acetaldehyde levels than did the ANA strain (C. Eriksson 1973). This difference, together with the finding that the AA animals had higher mitochondrial redox ratios than the ANA rats during ethanol intoxication, was explained by a faster mitochondrial oxidation of acetaldehyde in the AA animals, caused by higher mitochondrial aldehyde dehydrogenase (ALDH) activity. This explanation was confirmed later by Koivula et al. (1975) and Koivula and Lindros (1975), who found significantly less hepatic aldehyde utilization capacity and mitochondrial and microsomal ALDH activities in the ANA strain. These researchers also found strain differences in the opposite direction for the cytosolic aldehyde and alcohol dehydrogenase (ADH) activities. Obviously, the cytosolic ALDH activity was not regulatory for the acetaldehyde metabolism in these strains, which agrees with several recent findings suggesting the mitochondria as the main site for the oxidation of ethanol-derived acetaldehyde in rats (C. Eriksson et al. 1974; Grunnet 1973; Koivula and Koivusalo 1975; Lindros et al. 1972; Tottmar et al. 1973). Thus, even if the cytosolic ALDH activity may be of importance in some cases in modulating in vivo acetaldehyde levels, as has also been demonstrated (C. Eriksson et al. 1975; Petersen et al. 1977), the higher total hepatic ALDH activity, reflecting the microsomal and mitochondrial differences, caused the overall result of a lower acetaldehyde level in the AA strain. These findings are also in agreement with recent reports on negative individual correlations betwen hepatic acetaldehyde concentration and total ALDH activity (C. Eriksson 1977*a*, 1977*b*). Further confirmation of the correlation between ALDH activity, acetaldehyde concentrations, and alcohol preference was seen when the Astra diet was introduced in the F_{19} generation. This diet markedly reduced the drinking of all animals without affecting the strain differences (K. Eriksson and Närhi 1973). It was later found that this diet contained the ALDH inhibitor cyanamide (Marchner and Tottmar 1976) and that highly elevated blood and hepatic acetaldehyde concentrations after alcohol administration followed the consumption of this diet (Lindros et al. 1975).

The finding of higher cytosolic ALDH activity in the ANA strain still remains rather difficult to explain. Moreover, this finding is supported by a recent report by Berger and Weiner (1977), who found an extra cytosolic ALDH isoenzyme in their nondrinker rat population. One possible explanation would be that the cytosolic dehydrogenase activities (ADH and ALDH) somehow are linked. However, no conclusions can be drawn because of the lack of further data on this aspect.

Higher ADH activities, which increase the ethanol oxidation rate and consequently form acetaldehyde more rapidly, as suggested in the model of figure 2, may at first seem a paradox when compared with the earlier discussion of the faster ethanol metabolism found in the AA strain (figure 1). However, an explanation would be the following: In the ANA rats, which, in contrast to the AA strain, consume only small amounts of ethanol, the rate-limiting step becomes the ADH activity because the ADH system works below the K_m for ethanol estimated to 0.5-2.7 mM for rat liver ADH (Makar and Mannering 1970; Reynier 1969). Thus, those ANA animals with the highest ADH activity would also produce the highest acetaldehyde concentrations, and because of the aversive (conditioned from the habituation period) effect of the acetaldehyde, these animals would become selected for breeders within this strain. A positive correlation between ethanol oxidation rate and the hepatic acetaldehyde output has been demonstrated previously (C. Eriksson 1977*b*). In the AA case the ethanol oxidation rate is probably regulated by the general metabolic rate as discussed earlier.

A connection betwen ADH activity and alcohol drinking, opposite to that found in the AA and ANA rat strains, has been reported in mice (K. Eriksson and Pikkarainen 1968; Rodgers et al. 1963).

Figure 2. Possible Relationships Among Factors Suggested to be Associated with Ethanol Preference


SELECTION (for an aversive factor)
Liver ALDH ↓
Liver ADH ↑
Oxidation of acetaldehyde ↓
Oxidation of ethanol ↑
Liver acetaldehyde concentration ↑
Blood acetaldehyde concentration ↑
Inhibition of brain biogenic aldehyde metabolism ↑
Peripheral release of catecholamines ↑
Biogenic aldehyde concentration ↑
Peripheral toxic effect ↑
PREFERENCE ↓

However, because these differences were obtained by comparing inbred mice strains, the obtained correlations (with the higher ADH activity associated with higher alcohol preference) could be spurious, as discussed earlier in this chapter. This was also demonstrated by McClearn (1968), who tested the individual correlations between alcohol preference and ADH activity in an F_2 generation (obtained from the C57BL and DBA mice strain) and in a heterogeneous mouse population (HS). The result was a low significant correlation only in the HS population, indicating that the previously observed correlation between high ADH activity and ethanol drinking perhaps was more coincidental than of real importance in explaining the regulation of alcohol preference. Even if the ADH/ethanol preference relationship in mouse populations thus seems somewhat obscure, the possibility of species differences between rat and mouse in this respect cannot be totally excluded.

In contrast to the ADH aspect, there are a number of observations that all confirm the bond betwen ALDH activity, acetaldehyde, and alcohol preference as demonstrated in figure 2. One of the first observations which suggested that acetaldehyde levels may influence ethanol-drinking behavior was reported by Schlesinger et al. (1966), who found mouse strain differences, with the DBA mice displaying lower alcohol preference and higher blood acetaldehyde concentrations (after ethanol administration) than the C57BL strain. These investigators also found that disulfiram (Antabuse), an inhibitor of ALDH, increased the blood levels of acetaldehyde and caused a significant decrease in the alcohol preference. Several recent reports on drugs or diets that increase acetaldehyde levels and decrease ethanol preference support this relationship (Amit et al. 1976; K. Eriksson et al. 1975; Koe and Tennen 1970; Komura 1974; Sanders et al. 1977). Further evidence for the aversive role of acetaldehyde on ethanol drinking was found by Sheppard et al. (1968, 1970). They confirmed the earlier DBA-C57BL strain differences and found, in addition, that the acetaldehyde differences probably were caused by corresponding differences in the hepatic ALDH activities. These data were not supported by a similar type of study in which C57BL and CBA (another mouse strain with low alcohol preference) mice were compared (K. Eriksson and Pikkarainen 1970). However, these investigators measured cytosolic ALDH and, as already described, the mitochondrial ALDH activities are probably the main regulators of the acetaldehyde metabolism. Thus, these results do not necessarily provide evidence against the aversive role of acetaldehyde in these mouse strains. The lack of acetaldehyde, ALDH, and ADH differences between the nondrinker (ND) and drinker (D) rat strains of Li and Lumeng (1977) also cannot be taken as too strong evidence against a general aversive role of acetaldehyde because these strains were originally developed from only one pair of high-drinking and one pair of low-drinking Wistar rats (Lumeng et al. 1977). There is therefore no guarantee that the differences between the inbred strains derived from these pairs include all the factors regulating the alcohol consumption in the original heterogeneous population.

The most recent evidence for an aversive effect of acetaldehyde comes from a study with a Sprague-Dawley rat population in which negative individual correlations were found between blood acetaldehyde levels (after ethanol administration) and the ethanol preference with two different diets (C. Eriksson 1980). Moreover, the individual total ALDH activities were correlated negatively with the acetaldehyde levels and positively with the ethanol preference. Therefore, considering the AA and ANA findings, together with all other supporting data, it seems necessary to regard acetaldehyde as one aversive factor modulating voluntary alcohol consumption. In studies in which changes are induced in the drinking behavior, changes in the acetaldehyde metabolism (in addition to possible changes in the food intake) should be considered before other conclusions regarding the regulation of the drinking behavior are drawn.

Two main possibilities for the mechanism of acetaldehyde-induced aversion for ethanol preference are suggested in the model in figure 2. The first possibility includes an effect (perhaps toxic) associated with an acetaldehyde-induced peripheral release of catecholamines. Evidence for such an acetaldehyde effect has been reported by Perman (1958), Walsh et al. (1969), and Schneider (1971). The differences between AA and ANA rats (table 1), with the more depressed intracardiac conduction and the shorter duration of tachycardia in the ANA rats compared with the AA strain (Hillbom and Boguslawsky 1978), could perhaps be associated with these effects. Recent evidence for the acetaldehyde-induced catecholamine release effect was also provided by Nagasawa et al. (1977), who were successful in reversing this reaction with penicillamine, which binds the acetaldehyde. It is important to note that this mechanism probably also contributes to the extreme "alcohol sensitivity" observed in a portion of the Oriental population (Ewing et al. 1974; Ijiri 1974; Wolff 1972). The best evidence along this line is the positive individual correlation between blood acetaldehyde concentration and urine content of epinephrine or norepinephrine found in "alcohol sensitive" Orientals (Ijiri 1974). As indicated by Ewing et al. (1974), it also seems that this "alcohol sensitivity" could be a factor limiting alcohol consumption in these individuals.

The other main route for the aversive effect of acetaldehyde on ethanol drinking would be through the competitive inhibition of the biogenic amine metabolism in the brain as originally suggested by C. Eriksson (1973). Such an inhibition has been confirmed by in vitro data (Erwin and Deitrich 1966; Lahti and Majchrowicz 1969). Recent studies have shown negligible amounts of acetaldehyde in the brains of rats (C. Eriksson and Sippel 1977; Sippel 1974) and mice (Tabakoff et al. 1976), in spite of acetaldehyde concentrations up 200 μM in the circulating blood. This clearly supports the concept of a rapid acetaldehyde metabolism during the penetration into the brain from the blood, or from the cerebrospinal fluid where acetadehyde also has been found (Kiianmaa and Virtanen 1978; Pettersson and Kiessling 1977; Tsukamoto 1972). Thus, the brain ALDH (B-ALDH) will be the key enzyme with which acetaldehyde interacts. Subsequently, alterations of B-ALDH activity, by whatever means, should also affect alcohol preference. Convincing evidence that this can be the case was found in recent investigations by Amir (1977, 1978). This investigator found significant positive correlations between alcohol consumption and the hepatic ALDH activity, supporting the earlier described ALDH/acetaldehyde/preference relationship.

Another very recent finding supporting the importance of the B-ALDH activity in regulating ethanol preference has been reported by Sinclair and Lindros (1978). Their experimental design included first a phase of calcium cyanamide treatment during which the blood acetaldehyde was elevated after ethanol administration and the alcohol consumption decreased. After this period, 4-methylpyrazole treatment was added which abolished the increased blood acetaldehyde levels after ethanol administration but still kept the B-ALDH and alcohol preference inhibited.

Based on the probable mechanism of lower B-ALDH activity being responsible for lower alcohol consumption, it was surprising not to find any differences between these enzyme activities in the AA and ANA strains. The results for the AA and ANA B-ALDH total activities, measured from generation F_{28}, are given in table 2. Because we used acetaldehyde as substrate for the total B-ALDH determinations and Amir used indole-3-acetaldehyde bisulphite, it is possible that, even if his activities are of the same order as for the AA and ANA strains, his B-ALDH activities may more accurately reflect the functional B-ALDH capacity in regard to the catabolism of the biogenic amines. Another possibility would be that the B-ALDH activity is not associated with the acetaldehyde-induced AA-ANA preference differences. However, as will be discussed later in this chapter, there is a third, very intriguing possibility that may explain the lack of B-ALDH differences between the AA and ANA strains.

Table 2. Acetaldehyde Oxidation Capacity (B-ALDH)[1] of the Whole Brain Homogenate of the AA and ANA Strains

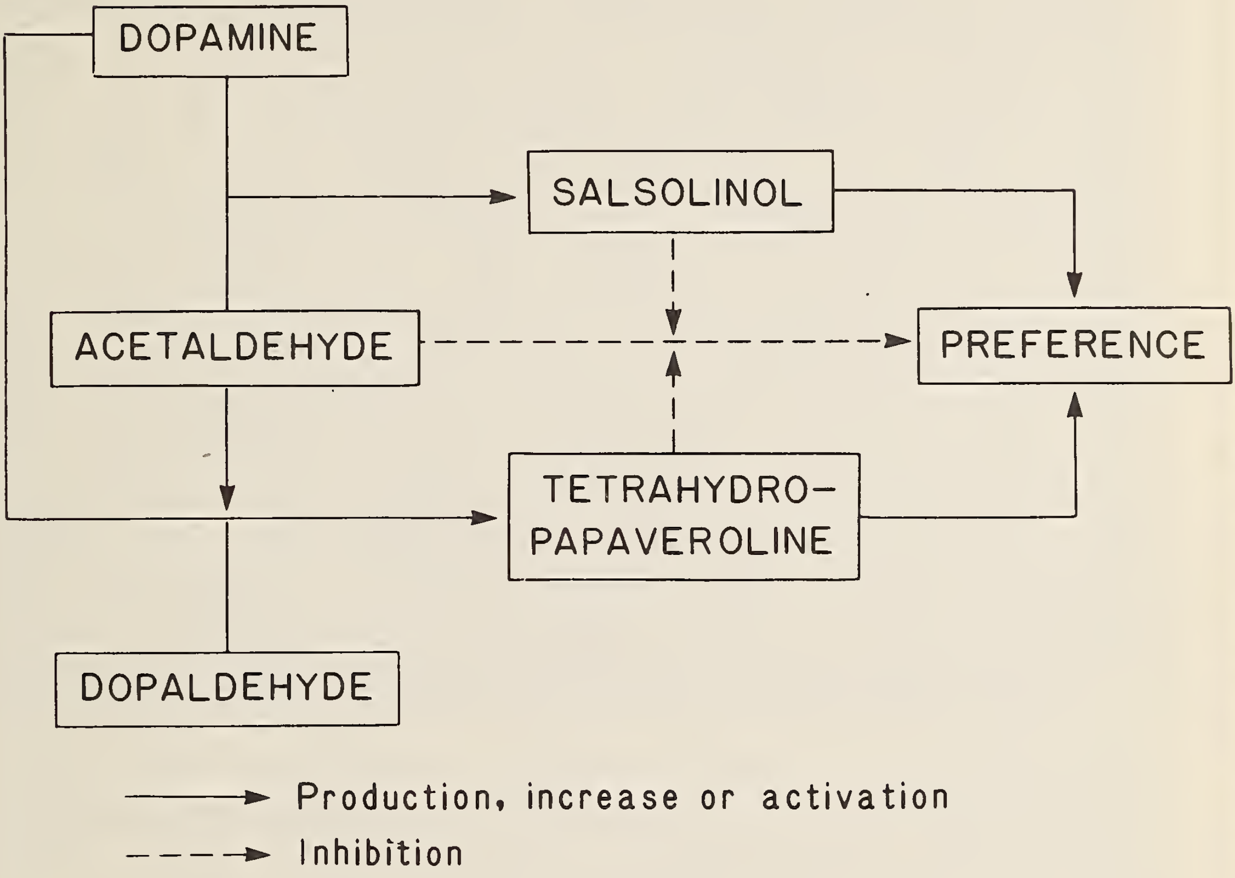
Strain	Sex	B-ALDH ± S.D. (μmol/min xg wet wt. of brain)	*N*
AA	M	0.106 ± 0.038	10
ANA	M	0.109 ± 0.022	10
AA	F	0.119 ± 0.011	10
ANA	F	0.110 ± 0.015	10

[1] The B-ALDH was measured by assessing the rate of NAD dependent AcH disappearance of whole homogenates, as described by Koivula et al. (1975) for the liver. Reaction rates were determined at 37° C in 50 mM Na-K^+ phosphate buffer, pH 7.4, supplemented with 4 mM NAD, 0.16 mM 4-methylpyrazole and an initial acetaldehyde concentration of 0.1 mM.

The acetaldehyde-induced competitive inhibition of the B-ALDH activity should in principle result in (at least) local increases in the biogenic aldehyde concentration followed by the subsequent increase in the ratio between alcohol and acid amine metabolites. This effect is suggested to modulate the overall increase of both the alcohol and acid amine metabolites, which is reported to be caused by the direct effect of ethanol, i.e., inhibition of transport from the brain (Tabakoff et al. 1975) and/or increased catecholamine turnover (Karoum et al. 1976). As suggested by Amir (1978), the increased endogenous aldehyde levels could become one possibility for creating the aversive effect on the alcohol preference, e.g., by inhibiting the Mg^{++}-dependent, (Na^+K^+)-activated ATPase (Erwin et al. 1975; Tabakoff 1974).

It seems that the acetaldehyde-induced aversion is one main (negative) factor explaining the AA and ANA strain differences in the regulation of alcohol consumption. However, this might represent just one side of the total relationship between the acetaldehyde metabolism and alcohol consumption. The other side of this aspect involves the original finding by Myers and Veale (1969), who discovered that intraventricular administration of acetaldehyde could reinforce ethanol drinking. Amit et al. (1979) recently reported that they also were able to reinforce subsequent ethanol drinking by the intraventricular administration of acetaldehyde. The recent reports

Figure 3. **Possible Mechanism for a Biphasic Effect of Acetaldehyde on Ethanol Preference**



of amine-aldehyde condensation products mediating the same effects (Myers and Melchior 1977*a*, 1977*b*) also support this idea. Thus, the effects of acetaldehyde could be biphasic in nature. The proposed model for this possibility is demonstrated in figure 3. It should be noted that the role of dopamine has been pointed out only as the most likely candidate. However, these pathways could also serve for other amines and their condensation products. It is possible that such a mechanism is also involved in the regulation of the high drinking behavior of the AA strain. In this case the absence of the B-ALDH differences between the strains would be explained, because low B-ALDH activity might favor the chronic action of the formation of the amine-aldehyde alkaloids in the AA strain as well as an acute aversive action in the ANA strain. The investigation of which of these relationships (if any) are real should be an interesting aspect of future alcohol preference research.

Relationship Between Alcohol Consumption and the Regulation of Alcohol Intoxication

The first evidence of an AA-ANA strain difference with regard to the degree of ethanol intoxication was noted by Rusi et al. (1977). These investigators observed a significantly longer sleep time in the male ANA rats compared with male AA rats after an intraperitoneal ethanol dose of 4 g/kg. With the female rats no significant differences were observed. These findings were supported by the results of Nikander and Pekkanen (1977), who discovered that the alcohol-induced impairment of the tilting plane performance was significantly greater in the ANA strain than in the AA animals. Only males were tested in these experiments. These differences could have been explained by an acetaldehyde-induced potentiation of the alcohol intoxication in the ANA strain. However, the experiments by Malila (1978) quite conclusively demonstrated that these strains differed in regard to some general mechanism for intoxication. This study demonstrated that isopropanol, tertiary butanol, and barbital (in addition to ethanol) impaired the tilting plane performance more in the ANA strain than in the AA strain. Thus, the explanatory value of acetaldehyde was reduced to mostly a contributory factor during ethanol intoxication. Also the new data obtained from the AT (alcohol tolerant strain) and ANT (alcohol nontolerant strain) selection study (table 9, K. Eriksson and Rusi, in this report) argue against any role for acetaldehyde in this aspect of ethanol action. These data, obtained from the AT and ANT strains (developed on the basis of the susceptibility to ethanol intoxication), support the negative correlation between voluntary ethanol consumption and alcohol intoxication in both sexes (table 10, K. Eriksson and Rusi, in this report). That increased intoxication correlates with decreased alcohol consumption is in agreement with numerous studies in which different drugs with their own intoxicating effects have been tested (see the review by Wallgren and Barry 1970).

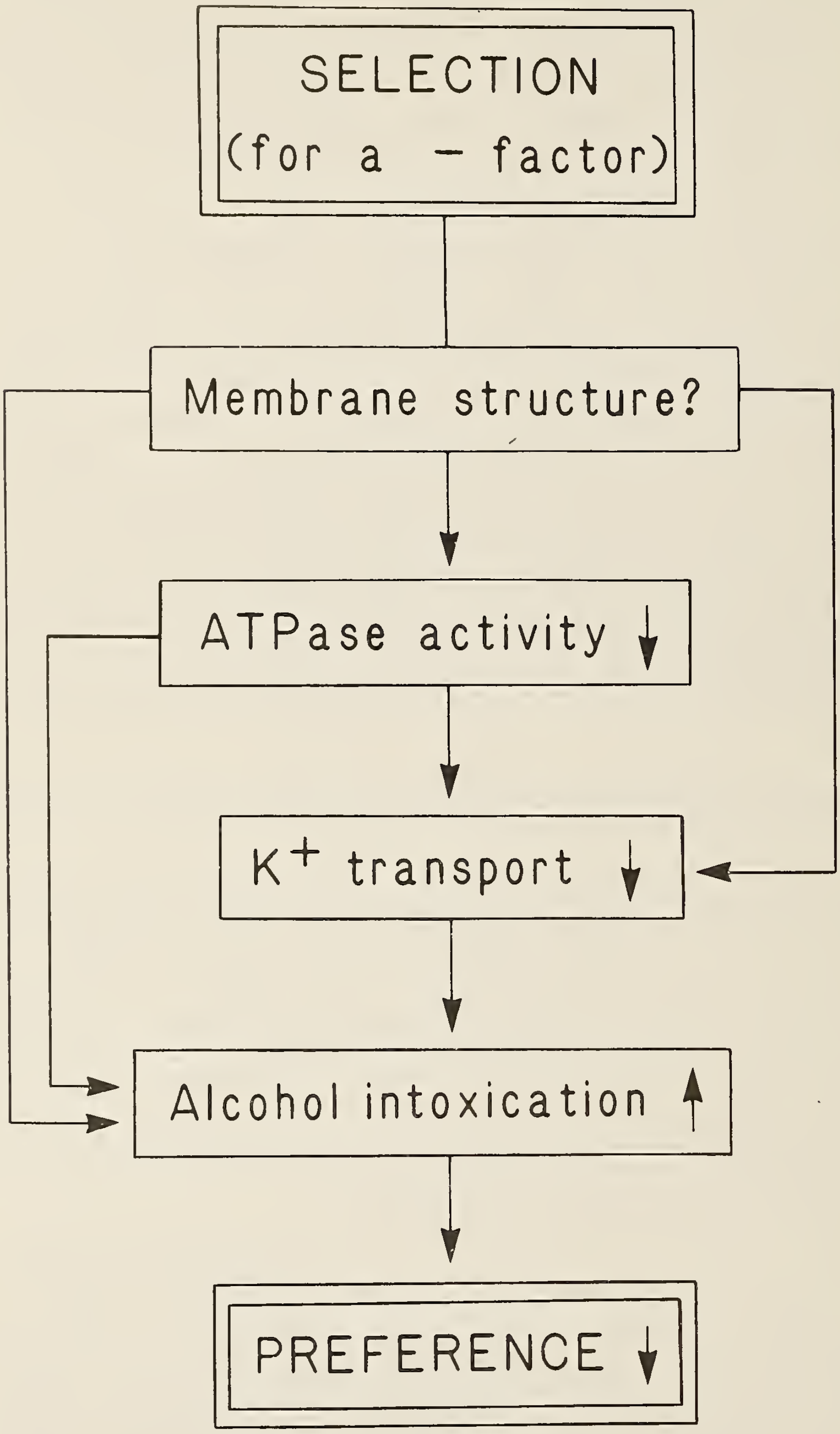
The proposed mechanism for how the animals could have been selected based on intoxication is outlined in figure 4. No evidence has been sought for differences in the membrane structure. This possibility is included because of the recent report that the primary effect causing intoxication could be an ethanol-induced membrane fluidity change (Seeman 1972). The reasons for the existence of the ATPase activity and the K^+-transport in the model (figure 4) are as follows: Significant innate strain differences, with the AA animals displaying higher brain microsomal $Mg^{++}(Na^+K^+)$ ATPase and Mg^{++}ATPase activities than the ANA strain, have been found by Nikander and Pekkanen (1977). The importance of this finding was neglected, mostly because the enzyme activities from the two strains were measured at different times and a subsequent study failed to observe significant differences. However, it is necessary to investigate the importance of these findings because these enzyme activities have been suggested to be related to the mechanism for intoxication (Israel et al. 1965; Järnefelt 1961). Moreover, this may also explain the K^+-transport differences between the strains. Boguslawsky and Nikander (1972) discovered that the recovery of the K^+-ions, after the ending of an electrical stimulation of cortex slices, was much more inhibited in the ANA animals than in the AA animals. This effect was later investigated in more detail (Nikander and Boguslawsky 1977). The inhibition of the active ion transport has been suggested to contribute to the mechanism by which ethanol depresses nerve function (Israel 1970; Israel et al. 1966). Thus, it seems that the AA and ANA strains have served well, not only in the evaluation of factors regulating alcohol preference but also as a tool in finding regulatory pathways for the mechanisms of ethanol intoxication.

Additional Characteristics Found in the AA and ANA Strains

In addition to the relationships described earlier in the text, strain differences have been found regarding open-field activity, urinary excretion, and the alcohol deprivation effect (table 1). The activity difference, with the ANA animals being more active than the AA animals, was found first in F_{17} (K. Eriksson 1972) and later confirmed in F_{20} (Rusi et al. 1977). The development of this strain difference has not been explained, partly because of the lack of knowledge about the CNS regulation of this emotional behavior.

The urinary excretion differences are also difficult to explain. The first study found that the ANA males displayed higher innate urinary excretion (after water intubation) and the females displayed lower urinary excretions (hydration and ethanol) than the corresponding sexes of the AA strain (Linkola 1976). The second study, without the prehydration procedure, demonstrated opposite differences with higher urinary excretions (urine output and Na^+) during alcohol intoxication in the ANA strain compared with the AA animals (Linkola et al. 1977). In this study the values from both sexes were pooled. The strain difference in the diuresis was explained by the higher vasopressin (antidiuretic hormone) excretion in the AA rats.

Figure 4. **A Possible Model of Selection for Ethanol Preference Based upon Intoxication**



The third study on this aspect (Linkola and Fyhrquist 1978) confirmed the results of the first study with similar innate differences, but revealed opposite differences in the vasopressin excretion compared with the results of the second study. In this third study, only males were used and Na^+ and K^+ excretions were not recorded. The fact that the animals in studies 1 and 3 were water loaded before the alcohol treatment could perhaps explain the contradictory results (Linkola et al. 1980). Moreover, Linkola and colleagues (1980) suggested that the high innate salt-seeking behavior of ANA rats associated with their high renal salt excretion leads them to use more likely energy sources with salt than those without salt. On the other hand, AA rats more likely consume ethanol because they cannot excrete salt from the food as effectively as ANA rats. Different thirst mechanisms in AA and ANA rats may also play some role in their drinking behavior (Linkola et al. 1980).

Sinclair (1979) discovered that the ANA animals displayed almost no alcohol deprivation effect (= ADE: transient increase in the alcohol consumption after deprivation period) in contrast to the AA rats. The AA rats have a normal ADE in magnitude, but it is more persistent than that of normal rats. When AA rats with the lowest E/T and ANA rats with the highest E/T were compared, (the E/T was now the same in both strains), the ADE strain difference still persisted, which suggests that the ADE difference was not an important factor in the regulation of the alcohol drinking, at least in the animals investigated. Still, as pointed out by Sinclair, because of the nature of this selection, the ADE differences should bear either a direct or an indirect relation to some factor regulating alcohol consumption, but which for some reason was not acting in the chosen animals. It is possible that the reason for this relates to the changes in the use of behavioral traits during the course of the selection. Because of the lack of further data, the possible relation between the ADE and the regulation of alcohol consumption cannot yet be fully evaluated.

Summary

Several metabolic, physiological, pharmacological, and behavioral differences between the nondrinking (ANA) and drinking (AA) outbred rat strains have been reported. Most of the strain differences seem to fit within the framework of one positive and two negative factors regulating alcohol consumption. The energy requirement (caloric need) is suggested to be the main positive factor. This factor would explain the higher general metabolic rate, higher rectal temperature, faster ethanol metabolism, higher food intake, lower food utilization, lower body and brain weights, and perhaps the higher whole brain serotonin and dopamine concentrations found in the AA rats.

The main negative factors are suggested to be the acetaldehyde and the intoxication factors. The acetaldehyde-induced aversion is suggested to be caused mainly by a peripheral toxicity effect but is also partly caused by the competitive inhibition of the brain aldehyde dehydrogenase activity. The acetaldehyde factor explains the lower hepatic acetaldehyde concentrations and perhaps the lower intracardiac conduction and the shorter duration of tachycardia in the ANA rats. The intoxication factor would explain the lower brain microsomal ATPase activity, the more disturbed K^+-transport in the cortex, and the higher degree of intoxication from various drugs in the ANA rats.

Because of the nature of this selection it is suggested that these regulatory factors also are of general importance for the regulation of alcohol drinking in experimental animals. Pulling out these three factors should not be taken as evidence that they are the only ones regulating voluntary alcohol intake, even in the AA and ANA strains. However, it is suggested that every investigation in which alcohol consumption is changed should, at least, be controlled for possible changes in food utilization, acetaldehyde metabolism, and intoxication before other conclusions are drawn about the regulation of the changed alcohol intake.

References

Ahtee, L., and Eriksson, K. 5-hydroxytryptamine and 5-hydroxyindolylacetic acid content in brain of rat strains selected for their alcohol intake. *Physiol Behav*, 8:123-126, 1972.

Ahtee, L., and Eriksson, K. Regional distribution of brain 5-hydroxytryptamine in rat strains selected for their alcohol intake. *Ann NY Acad Sci*, 215:126-134, 1973.

Ahtee, L., and Eriksson, K. Dopamine and noradrenaline content in the brain of rat strains selected for their alcohol intake. *Acta Physiol Scand*, 93:563-565, 1975.

Amir, S. Brain and liver aldehyde dehydrogenase: Relations to ethanol consumption in Wistar rats. *Neuropharmacology*, 16:781-784, 1977.

Amir, S. Brain and liver aldehyde dehydrogenase activity and voluntary ethanol consumption by rats: Relations to strain, sex and age. *Psychopharmacology*, 57:97-102,1978.

Amit, Z.; Brown, Z.W.; Rockman, G.; Smith, B.; and Amir, S. Acetaldehyde: A positive reinforcer mediating ethanol consumption. In: Begleiter, H., ed. *The Biological Effects of Alcohol*. New York: Plenum Press, 1979.

Amit, Z.; Levitan, D.E.; and Lindros, K.O. Suppression of ethanol intake following administration of dopamine-beta-hydroxylase inhibitors in rats. *Arch Int Pharmacodyn Ther*, 223:114-119, 1976.

Berger, D., and Weiner, H. Relationship between alcohol preference and biogenic aldehyde metabolizing enzymes in rats. *Biochem Pharmacol*, 26:841-846, 1977.

Boguslawsky, P., and Nikander, P. A difference between high-and-low-drinking rats in effects of ethanol on ion movements in cerebral tissue. *Acta Physiol Scand*, 84:12A-13A, 1972.

Collins, A.C. Genetics of ethanol preference: Role of neurotransmitters. In: Majchrowicz, E., and Noble, E.P., eds. *Biochemistry and Pharmacology of Ethanol.* Vol. 2. New York: Plenum Press, 1979. pp. 207-221.

Eriksson, C.J.P. Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochem Pharmacol*, 22:2283-2292, 1973.

Eriksson, C.J.P. The distribution and metabolism of acetaldehyde in rats during ethanol oxidation. II. Regulation of the hepatic acetaldehyde level. *Biochem Pharmacol*, 26:249-252, 1977*a*.

Eriksson, C.J.P. The aversive effect of acetaldehyde on alcohol drinking behavior in the rat. *Alcohol Clin Exp Res*, 4:107-111, 1980.

Eriksson, C.J.P. Regulation of acetaldehyde metabolism during ethanol oxidation in perfused rat liver. In: Gross, M.M., ed. *Alcohol Intoxication and Withdrawal.* Vol. IIIa. (*Vol. 85A of Advances in Experimental Medicine and Biology.*) New York: Plenum Press, 1977*b*. pp. 225-236.

Eriksson, C.J.P., and Sippel, H.W. The distribution and metabolism of acetaldehyde in rats during ethanol oxidation. I. The distribution of acetaldehyde in liver, brain, blood and breath. *Biochem Pharmacol*, 26:241-247, 1977.

Eriksson, C.J.P.; Lindros, K.O.; and Forsander, O.A. 2,4-Dinitrophenol-induced increase in ethanol and acetaldehyde oxidation in the perfused rat liver. *Biochem Pharmacol*, 23:2193-2195, 1974.

Eriksson, C.J.P.; Marselos, M.; and Koivula, T. Role of cytosolic rat liver aldehyde dehydrogenase in the oxidation of acetaldehyde during ethanol oxidation *in vivo*. *Biochem J*, 152:709-712, 1975.

Eriksson, K. Genetic selection for voluntary alcohol consumption in the albino rat. *Science*, 159:739-741, 1968.

Eriksson, K. Factors affecting voluntary alcohol consumption in the albino rat. *Ann Zool Fenn*, 6:227-265, 1969.

Eriksson, K. Rat strains specially selected for their voluntary ethanol consumption. *Ann Med Exp Biol Fenn*, 49:67-72, 1971.

Eriksson, K. Behavioral and physiological differences among rat strains specially selected for their alcohol consumption. *Ann NY Acad Sci*, 197:32-41, 1972.

Eriksson, K. Alcohol inhibition and behavior: A comparative study. In: Eleftheriou, B.E., ed. *Psychopharmacogenetics.* New York: Plenum Press, 1975. pp. 127-168.

Eriksson, K., and Malmström, K.K. Sex differences in consumption and elimination of alcohol in albino rats. *Ann Med Exp Biol Fenn*, 45:389-392, 1967.

Eriksson, K., and Närhi, M. Specially selected rat strains as a model of alcoholism. In: Spiegel, A., ed. *The Laboratory Animal in Drug Testing: Proceedings of the 5th ICLA Symposium.* Stuttgart: Gustav Fischer Verlag, 1973. pp. 163-171.

Eriksson, K., and Pikkarainen, P.H. Differences between the sexes in voluntary alcohol consumption and liver ADH-activity in inbred strains of mice. *Metabolism*, 17:1037-1042, 1968.

Eriksson, K., and Pikkarainen, P.H. Strain and sex differences in voluntary alcohol consumption, liver ADH activity and aldehyde-oxidizing capacity in inbred strains of mice. *Jpn J Stud Alcohol*, 5:1-7, 1970.

Eriksson, K.; Pekkanen, L.; Forsander, O.; and Ahtee, L. The effect of dietary factors on voluntary ethanol intake in the albino rat. In: Sinclair, J.D., and Kiianmaa, K., eds. *The Effects of Centrally Active Drugs on Voluntary Alcohol Consumption.* Vol. 24. Satellite Symposium to the Sixth International Congress of Pharmacology, Helsinki, July 26, 1975. Helsinki: Finnish Foundation for Alcohol Studies, 1975. pp. 15-26.

Erwin, V.G., and Dietrich, R.A. Brain aldehyde dehydrogenase: Localization, purification, and properties. *J Biol Chem*, 241:3533-3539, 1966.

Erwin, V.G.; Kim, J.; and Anderson, A.D. Effects of aldehyde on sodium plus potassium ion-stimulated adenosine triphosphatase of mouse brain. *Biochem*

Ewing, J.A.; Rouse, B.A.; and Pellizzari, E.D. Alcohol sensitivity and ethnic background. *Am J Psychiatry*, 131:206-210, 1974.

Fernström, J.D., and Wurtman, R.J. Brain serotonin content: Physiological regulation by plasma neutral amino acids. *Science*, 178:414-416, 1972.

Forsander, O.A., and Eriksson, C.J.P. Metabolic characteristics of rat strains consuming different amounts of alcohol. In: Forsander, O.A., and Eriksson, K., eds. *International Symposium on Biological Aspects of Alcohol Consumption.* Vol. 20. Helsinki: Finnish Foundation for Alcohol Studies, 1972. pp. 43-49.

Forsander, O.A., and Eriksson, K., eds. *International Symposium on Biological Aspects of Alcohol Consumption.* Vol. 20. Helsinki: Finnish Foundation for Alcohol Studies, 1972.

Forsander, O.A., and Salaspuro, M. Voluntary ethanol consumption as related to ketone bodies metabolism. *Life Sci*, 9:467-470, 1962.

Grunnet, N. Oxidation of acetaldehyde by rat liver mitochondria in relation to ethanol oxidation and the transport of reducing equivalents across the mitochondrial membrane. *Eur J Biochem*, 35:236-243, 1973.

Hillbom, M.E., and Boguslawsky, K. Effect of ethanol on cardiac function in rats genetically selected for their ethanol preference. *Pharmacol Biochem Behav*, 8:609-614, 1978.

Ijiri, I. Studies on the relationship between the concentrations of blood acetaldehyde and urinary catecholamine and the symptoms after drinking alcohol. *Jpn J Stud Alcohol*, 9:35-59, 1974.

Israel, Y. Cellular effects of alcohol: A review. *Q J Stud Alcohol*, 31:293-316, 1970.

Israel, Y.; Kalant, H.; and Laufer, I. Effects of ethanol on Na, K, Mg-stimulated microsomal ATPase activity. *Biochem Pharmacol*, 14:1803-1814, 1965.

Israel, Y.; Kalant, H.; and LeBlanc, A.E. Effects of lower alcohols on potassium transport and microsomal adenosine triphosphatase activity of the rat cerebral cortex. *Biochem J*, 100:27-33, 1966.

Järnefelt, J. A possible mechanism of action of ethyl alcohol on the central nervous system. *Ann Med Exp Biol Fenn*, 39:267-272, 1961.

Karoum, F.; Wyatt, R.J.; and Majchrowicz, E. Brain concentrations of biogenic amine metabolites in acutely treated and ethanol-dependent rats. *B J Pharmacol*, 56:403-411, 1976.

Kiianmaa, K. Evidence for involvement of noradrenaline and against 5-hydroxytryptamine neurons in alcohol consumption by rats. In: Sinclair, J.D., and Kiianmaa, K., eds. *The Effects of Centrally Active Drugs on Voluntary Alcohol Consumption.* Vol. 24. Satellite Symposium to the Sixth International Congress of

Pharmacology, Helsinki, July 26, 1975. Helsinki: Finnish Foundation for Alcohol Studies, 1975. pp. 73-84.

Kiianmaa, K., and Virtanen, P. Ethanol and acetaldehyde levels in cerebrospinal fluid during ethanol oxidation in the rat. *Neurosci Lett*, 10:181-186, 1978.

Koe, B.K., and Tennen, S.S. Inhibiting action of n-butyraldoxime on ethanol metabolism and on natural ethanol preference of C57BL mice. *J Pharmacol Exp Ther*, 174:434-449, 1970.

Koivula, T., and Koivusalo, M. Different forms of rat liver aldehyde dehydrogenase and their subcellular distribution. *Biochim Biophys Acta*, 397:9-23, 1975.

Koivula, T., and Lindros, K.O. Effects of long-term ethanol treatment on aldehyde and alcohol dehydrogenase activities in rat liver. *Biochem Pharmacol*, 24:1937-1942, 1975.

Koivula, T.; Koivusalo, M.; and Lindros, K.O. Liver aldehyde and alcohol dehydrogenase activities in rat strains genetically selected for their ethanol preference. *Biochem Pharmacol*, 14:1807-1811, 1975.

Komura, S. Effects of ethyleneglycol dinitrate and related compounds on ethanol preference and ethanol metabolism. *Acta Pharmacol Toxicol*, 35:145-154, 1974.

Lahti, R.A., and Majchrowicz, E. Acetaldehyde: An inhibitor of the enzymatic oxidation of 5-hydroxyindoleacetaldehyde. *Biochem Pharmacol*, 18:535-538, 1969.

Li, T.-K., and Lumeng, L. Alcohol metabolism of inbred strains of rats with alcohol preference and nonpreference. In: Thurman, R.G.; Williamson, J.R.; Drott, H.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems. Vol. III. Intermediary Metabolism and Neurochemistry*. New York: Academic Press, 1977. pp. 625-633.

Lindros, K.O.; Koivula, T.; and Eriksson, C.J.P. Acetaldehyde levels during ethanol oxidation: A diet-induced change and its relation to liver aldehyde dehydrogenases and redox states. *Life Sci*, 17:1589-1598, 1975.

Lindros, K.O.; Vihma, R.; and Forsander, O.A. Utilization and metabolic effects of acetaldehyde and ethanol in the perfused rat liver. *Biochem J*, 126:945-952, 1972.

Linkola, J. Urine sodium, potassium and osmolality in two rat strains selected for their different ethanol preferences. *Med Biol*, 54:254-259, 1976.

Linkola, J., and Fyhrquist, F. Urinary cyclic AMP and vasopressin excretion in rat strains selected for their alcohol intake. *Acta Physiol Scand*, 102:364-367, 1978.

Linkola, J.; Fyhrquist, F.; and Forsander, O. Effects of ethanol on urinary arginine vasopressin excretion in two rat strains selected for their different ethanol preferences. *Acta Physiol Scand*, 101:126-128, 1977.

Linkola, J.; Tikkanen, I.; Fyhrquist, F.; and Rusi, M. Renin, water drinking, salt preference and blood pressure in alcohol preferring and alcohol avoiding rats. *Pharmacol Biochem Behav*, 12:293-296, 1980.

Lumeng, L.; Hawkins, T.D.; and Li, T.-K. New strains of rats with alcohol preference and nonpreference. In: Thurman, R.G.; Williamson, J.R.; Drott, H.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems. Vol. III. Intermediary Metabolism and Neurochemistry*. New York: Academic Press, 1977. pp. 537-544.

Makar, A.B., and Mannering, G.J. Kinetics of ethanol metabolism in the intact rat and monkey. *Biochem Pharmacol*, 19:2017-2022, 1970.

Malila, A. Intoxication effects of three aliphatic alcohols and barbital on two rat strains genetically selected for their ethanol intake. *Pharmacol Biochem Behav*, 8:197-201, 1978.

Marchner, H., and Tottmar, O. Inhibition of the acetaldehyde dehydrogenases in rat liver by a cyanamide derivative present in a commercial standard diet for small animals. *Acta Pharmacol Toxicol*, 39:331-343, 1976.

McClearn, G.E. Genetics and motivation of the mouse. In: Arnold, W.D., ed. *Nebraska Symposium on Motivation*. Lincoln: University of Nebraska Press, 1968. pp. 47-83.

Myers, R.D., and Melchior, C.L. Alcohol drinking: Abnormal intake caused by tetrahydropapaveroline in brain. *Science*, 196:544-556, 1977*a*.

Myers, R.D., and Melchior, C.L. Differential actions on voluntary alcohol intake of tetrahydroisoquinolines or a β-carboline infused chronically in the ventricle of the rat. *Pharmacol Biochem Behav*, 7:381-392, 1977*b*.

Myers, R.D., and Veale, W.L. Alcohol preference in the rat: Reduction following depletion of brain serotonin. *Science*, 160:1469-1471, 1968.

Myers, R.D., and Veale, W.L. Alterations in volitional alcohol intake produced in rats by chronic intraventricular infusions of acetaldehyde, paraldehyde or methanol. *Arch Int Pharmacodyn Ther*, 180:100-113, 1969.

Nagasawa, H.T.; Alexander, C.S.; Goon, D.J.W.; and DeMaster, E.G. Lowering of blood acetaldehyde levels as a therapeutic approach to alcoholism. In: Thurman, R.D.; Williamson, J.R.; Drott, H.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems. Vol. III. Intermediary Metabolism and Neurochemistry*. New York: Academic Press, 1977. pp. 529-536.

Nikander, P., and Boguslawsky, P. A difference in the effects of ethanol on ion movements in cerebral tissue slices from alcohol preferring and alcohol avoiding rats. *TIT J Life Sci*, 7:1-6, 1977.

Nikander, P., and Pekkanen, L. An inborn alcohol tolerance in alcohol-preferring rats. The lack of relationship between tolerance to ethanol and the brain microsomal (Na^+K^+) ATPase activity. *Psychopharmacology*, 51:219-223, 1977.

Opitz, K. Beobachtungen bei Alcohol trinkenden Ratten—Einfluss von Fenfluramin. *Pharmakopsychiatr Neuropsychopharmakol*, 2:202-205, 1969.

Perman, E.S. The effect of acetaldehyde on the secretion of adrenaline and noradrenaline from the suprarenal gland of the cat. *Acta Physiol Scand*, 43:71-76, 1958.

Petersen, D.R.; Collins, A.C.; and Deitrich, R.A. Role of liver cytosolic aldehyde dehydrogenase isozymes in control of blood acetaldehyde concentrations. *J Pharmacol Exp Ther*, 201:471-481, 1977.

Pettersson, H., and Kiessling, K-H. Acetaldehyde occurrence in cerebrospinal fluid during ethanol oxidation in rats and its dependence on the blood level and on dietary factors. *Biochem Pharmacol*, 26:237-240, 1977.

Reynier, M. Pyrazole inhibition and kinetic studies of ethanol and retinol oxidation catalyzed by rat liver alcohol dehydrogenase. *Acta Chem Scand*, 23:1119-1129, 1969.

Rodgers, D.A.; McClearn, G.E.; Bennett, E.L.; and Hebert, M. Alcohol preference as a function of its caloric utility in mice. *J Comp Physiol Psychol*, 56:666-672, 1963.

Rusi, M.; Eriksson, K.; and Mäki, J. Genetic differences in the susceptibility to acute ethanol intoxication in selected rat strains. In: Gross, M.M., ed. *Alcohol Intoxication and Withdrawal*. Vol. IIIa. (*Vol. 85A of Advances in Experimental Medicine and Biology*.) New York: Plenum Press, 1977. pp. 97-109.

Sanders, B.; Collins, A.C.; Petersen, D.R.; and Fish, B.S. Effects of three monoamine oxidase inhibitors on ethanol preference in mice. *Pharmacol Biochem Behav*, 6:319-324, 1977.

Schlesinger, K.; Kakihana, R.; and Bennett, E.L. Effects of tetrathylthiuramdisulfide (Antabuse) on the mechanism and consumption of ethanol in mice. *Psychosom Med,* 28:514-520, 1966.

Schneider, F.H. Acetaldehyde-induced catecholamine secretion from the cow adrenal medulla. *J Pharmacol Exp Ther,* 177:109-118, 1971.

Seeman, P. The membrane actions of anaesthetics and tranquilizers. *Pharmacol Rev,* 24:583-655, 1972.

Sheppard, J.R.; Albersheim, P.; and McClearn, G.E. Enzyme activities and ethanol preference. *Biochem Genet,* 2:205-212, 1968.

Sheppard, J.R.; Albersheim, P.; and McClearn, G.E. Aldehyde dehydrogenase and ethanol preference in mice. *J Biol Chem,* 245:2876-2882, 1970.

Sinclair, J.D. Alcohol-deprivation effect in rats genetically selected for their ethanol preference. *Pharmacol Biochem Behav,* 10:597-602, 1979.

Sinclair, J.D., and Lindros, K.O. "Suppression of Alcohol Drinking by Aldehyde Dehydrogenase Inhibition: Its Independence of Acetaldehyde." Paper presented at the 1st Finnish Symposium on the Biological and Medical Effects of Alcohol, Hensinki, 1978.

Sippel, H.W. The acetaldehyde content in rat brain during ethanol metabolism. *J Neurochem,* 23:451-452, 1974.

Stricker, E.M., and Zigmond, M.J. Effects on homeostasis of intraventricular injections of 6-hydroxydopamine in rats. *J Comp Physiol Psychol,* 86:973-994, 1974.

Tabakoff, B. Inhibition of sodium, potassium and magnesium activated ATPase by acetaldehyde and "biogenic" aldehydes. *Res Commun Chem Pathol Pharmacol,* 7:621-624, 1974.

Tabakoff, B.; Anderson, R.A.; and Ritzmann, R.F. Brain acetaldehyde after ethanol administration. *Biochem Pharmacol,* 25:1305-1309, 1976.

Tabakoff, B.; Ritzmann, R.F.; and Boggan, W.O. Inhibition of the transport of 5-hydroxyindoleacetic acid from brain by ethanol. *J Neurochem,* 24:1043-1051, 1975.

Tottmar, S.O.C.; Pettersson, H.; and Kiessling, K.H. The subcellular distribution and properties of aldehyde dehydrogenases in rat liver. *Biochem J,* 135:577-586, 1973.

Tsukamoto, S. Studies on the distribution and the metabolism of alcohol in various internal organs and body fluid. 2. Distribution of ethanol and acetaldehyde in organs and humors of the guinea pig. *Jpn Stud Alcohol,* 7:168-175, 1972.

Wallgren, H., and Barry, H., III. Voluntary selection of alcohol. In: Wallgren, H., and Barry, H., III., eds. *Actions of Alcohol. Vol 2. Chronic and Clinical Aspects.* New York: Elsevier, 1970. pp. 403-477.

Walsh, M.J.; Hollander, P.B.; and Truitt, E.B., Jr. Sympathomimetic effects of acetaldehyde on the electrical and contractile characteristics of isolated left atria of guinea pigs. *J Pharmacol Exp Ther,* 167:173-186, 1969.

Wolff, P.H. Ethnic differences in alcohol sensitivity. *Science,* 175:449-450, 1972.

Selective Breeding for Ethanol Sensitivity: Short-Sleep and Long-Sleep Mice

Gerald E. McClearn and Ryoko Kakihana

After the initial demonstration of differences among inbred strains of mice in alcohol preference (McClearn and Rodgers 1959), numerous studies were devoted to exploring possible correlates of these consumption differences. One hypothesis was that the differences in voluntary ingestion might be related to differences in susceptibility of the animals to the effects of alcohol. The initial approach to assessing this hypothesis was to measure the effects of forced inhalation of ethanol vapor on the activity of several inbred strains (McClearn 1962). In brief, alcohol was found to have a depressant effect on activity in both C57BL and DBA/2 mice. Because these were the most extreme strains with respect to preference, with the C57BLs showing high consumption and DBA/2s showing almost complete avoidance of ethanol, the results appeared to invalidate the naive hypothesis. Nevertheless, the results were interesting, because two strains (the RIII and the BALB/c) were unaffected by the treatment and another strain (the C3H) showed an increase in activity. Thus, at least by this mode of administration, with the dose used in this study, and with activity measured in this fashion, strain differences were observed even though they could not be easily related to differences in voluntary consumption levels of the strains.

Meanwhile, another approach was being used in which dosage could be better controlled. Kakihana et al. (1966) found the duration of loss of the righting response ("sleep time," in laboratory parlance) subsequent to an intraperitoneal injection of 3.3 g ethanol/kg body weight to be strikingly different in C57BL and BALB/c mice. The direction of this difference was compatible with the hypothesis of an association between sensitivity and avidity: The C57BLs were less affected by this soporific dose than were the BALB/c animals.

Limitations on the interpretation of findings of associated characters in two inbred strains (see McClearn 1979) were recognized, of course. We therefore undertook a selective breeding study with the expectations that we could derive lines differing very substantially with respect to ethanol sensitivity as measured by sleep time and that these lines would provide useful tools for later investigations of the mechanisms of ethanol's narcotic effect.

Foundation Stock

The foundation stock comprised 44 male and 53 female mice of the first generation of the HS population. This genetically heterogeneous stock, whose origins and maintenance were described by McClearn et al. (1970), was derived from intercrosses of eight inbred strains: A, AK, BALB/c, C3H, C57BL, DBA/2, Is/Bi, and RIII. The stock is maintained by restricted random mating, with sib matings absolutely proscribed and cousin matings avoided when possible.

Method

Animals used in the selective breeding program are maintained in standard cages with like-sexed littermates from time of weaning until testing. For the first 13 generations, weaning occurred at 28 days of age and the animals were tested when they were 72 days old; from generation 14 to the present, weaning and testing have occurred at 25 and 60 days of age, respectively. A 12-hour light-dark cycle is maintained, and testing is carried out during daylight hours.

The procedure initially was as follows: Immediately after an intraperitoneal ethanol injection (3.3 g/kg), each animal was placed on the inside wall of a 1/2-inch wire mesh cylinder, 10 inches in diameter, closed at the top by a wooden circle, and supported 14 inches above a foam rubber mat by a central wooden dowel. The time from injection until the animal fell to the mat was recorded as "fall time." The mouse was then placed on its back in a 12-inch-long, V-shaped trough so that duration of loss of the righting response could easily be measured. The top edges of the V were 2 3/4 inches apart, and the sides met at an angle of approximately 90 degrees. Formed plastic troughs mounted on arms that activated timers were introduced in generation 8 (G_8) to facilitate timing.

Also in G_8, the fall time measure was replaced by a "restraint time" procedure. After injection, the animal was placed in a clear plastic cylinder approximately 1 inch in diameter. The forward end of the cylinder was sealed, and the end through which the animal was introduced was blocked by a gate. Ventilation holes were provided. The dimensions of the cylinder prevented the animal from gross bodily movement during the restraint period. After 1 minute, the cylinder was inverted and the animal gently drawn by its tail until it rested on its back in the trough. It was later established in other research that an experienced observer could judge quite accurately when the righting response had been lost simply by watching the animal in a standard cage. This alternative restraint time procedure has therefore been used since G_{25}.

In all procedures, the mouse was judged not to have lost the righting response if it righted itself within 1 minute after being placed in the trough. It was then returned to the foam mat (fall time) or to the restraining cylinder or standard cage (restraint time) for an additional 30 seconds before being placed again in the trough. This procedure was repeated as necessary until the righting response was lost or until 10 minutes had passed. In the latter event, the animal was assigned a score of 600 seconds for fall time or restraint time and a score of zero for "sleep time."

The procedure for determining recovery of the righting response was as follows for G_0 through G_5: Thirty seconds after the animal first righted itself, it was again placed on its back in the trough and the time was noted. If it righted itself within 30 seconds, it was returned to an inverted position. This test was repeated as necessary. The criterion for recovery was regaining the righting response three times in three consecutive, 30-second periods after the initial righting response. If the animal met this criterion, the time of the initial righting response was recorded as the end of sleep time. If it failed to right itself within 30 seconds of the initial righting response, it was regarded *not* to have met the recovery criterion and the procedure was repeated until the criterion was attained. It was observed during the first five generations of selection that if a righting response did occur during a 30-second period, it usually occurred within the first 15 seconds. Therefore, during subsequent generations, four consecutive, 15-second periods have been used to define recovery. Throughout the course of the selective breeding program, sleep time has been recorded as the time during which the righting response is absent as defined, and it has been the sole criterion for selection.

The dose of ethanol administered through G_7 was 3.3 g/kg of a 30 percent (v/v) ethanol solution made with physiological saline. A substantial number of the short-sleep (SS) animals failed to lose the righting response in that generation, and the dose was increased to 3.5 g/kg in G_8. When the effect of that increase was found to be minimal, the dose was increased to 4.2 g/kg in the next generation. That dosage was used from G_9 through G_{25}, when the number of zero scores in the SS line and the marked debilitating effects in the long-sleep (LS) line prompted a differential change in dose to 4.7 g/kg for the former and 3.8 g/kg for the latter.

Mass selection has been employed throughout the selective breeding program, with mates assigned at random but with a restriction against sib mating. The number of matings contributing to each generation and the number of animals tested in each generation are shown in table 1.

The study was initiated in 1962 in the laboratories of the Department of Psychology, University of California, Berkeley. Generations 4 and 5 were maintained and tested in the animal facilities of the Stanford University Medical School. In 1967, G_5 animals were shipped to their present location at the Institute for Behavioral Genetics, University of Colorado, Boulder. A drastic reduction in fertility accompanied the move, and the number of fertile matings in G_6 and G_7 was very low (see table 1). Selection was completely relaxed from G_{19} through G_{24}. Animals of G_{25} were tested and selection was resumed. A distinction is made in the following discussion between generations (G) and selected generations (S). In G_{18} (S_{16}), a sample of 150 HS animals was tested contemporaneously with the selected lines.

Results and Discussion

The distribution of sleep time scores for the foundation stock is shown at the top of figure 1. Although some degree of skewness is apparent, the distribution is reasonably normal. Although not requisite, a distribution of this shape is desirable for selection, suggesting

Table 1. Number of Families and Individuals in Consecutive Generations of Sleep Time Selection Study

Generation[1]		Short Sleep		Heterogeneous Stock		Long Sleep	
G	S	Number of Families	Number of Individuals	Number of Families	Number of Individuals	Number of Families	Number of Individuals
0	0			26	97		
1	1	8	59			5	29
2	2	6	44			3	32
3	3	8	52			11	85
4	4	8	95			10	75
5	5	13	99			11	84
6		5	19			1	3
7		3	43			1	23
8	6	6	54			8	81
9	7	9	66			8	71
10	8	9	85			7	45
11	9	9	64			8	49
12	10	9	127			9	170
13	11	10	125			9	85
14	12	10	171			11	170

Table 1. Number of Families and Individuals in Consecutive Generations of Sleep Time Selection Study

Generation[1]		Short Sleep		Heterogeneous Stock		Long Sleep	
G	S	Number of Families	Number of Individuals	Number of Families	Number of Individuals	Number of Families	Number of Individuals
15	13	10	156			9	145
16	14	8	142			9	86
17	15	8	157			6	129
18	16	11	160	24	150	10	156
19		13	133			5	48
20		16	133			15	119
21		12	85			12	85
22		12	91			12	96
23		11	92			9	74
24		8	42			8	59
25	17	9	123			5	63

[1] G refers to generations numbered consecutively from the beginning of the selection study. S refers to selected generations (i.e., not including those generations during which selection was relaxed).

that discriminations can be made among individuals in both tails of the distribution.

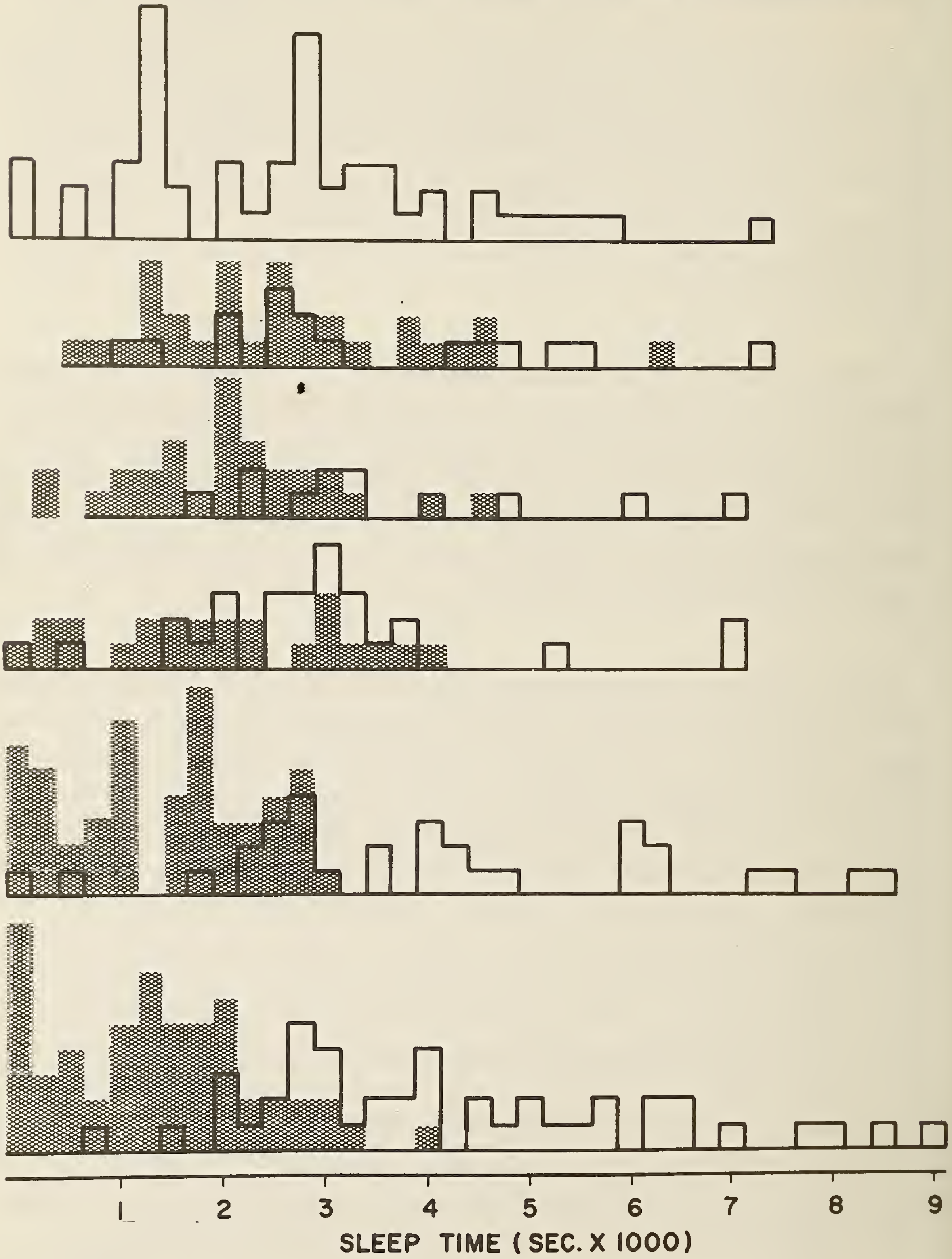
Figure 1 also shows the gradual divergence of the LS and SS distributions over the first five generations of selection. The means of generations S_0 through S_{17} are plotted separately by sex and line in figure 2. Examination of figures 1 and 2 reveals that the response to selection from S_0 through S_5 was quite symmetrical. Over this span of generations, realized heritability was calculated to be 0.18 (McClearn and Kakihana 1973).

Figure 2 also records the changes in ethanol dose that were made during the course of the study. The increase in sleep time in both lines from S_6 to S_7 is attributable to the change from 3.5 g/kg to 4.2 g/kg. The fact that response to selection continued in both lines after S_7 demonstrates clearly that the inbreeding imposed by the small breeding populations in G_6 and G_7 did not result in the elimination of relevant additive genetic variance.

The locations of the sleep time means for male and female HS mice tested contemporaneously with S_{16} (see figure 2) were at about the same levels as in the foundation stock, but this similarity cannot be interpreted strongly because of the change in dosage. These S_{16} control values do serve to indicate the cumulative asymmetry of response to that point. Although possible interpretations in terms of directional dominance or unequal selection pressures in the two directions might be invoked, the results are likely due, at least in part, to the fact that the mean of the SS line is nearly at the bottom limit of the measurement scale. This scalar constraint is clearly revealed in figure 3, which illustrates the frequency distributions of the two lines in S_{17} (G_{25}). The SS distribution is markedly skewed to the right, whereas the LS scores are still broadly distributed. That the means of neither line became less extreme after the period of relaxed selection between S_{16} and S_{17} indicates that the artificial selection in either direction has not been strongly opposed by natural selection.

The fall time measure might be expected a priori to be an indicant of the same physiological domain as sleep time. If so, a correlated fall time response should have occurred along with the sleep time

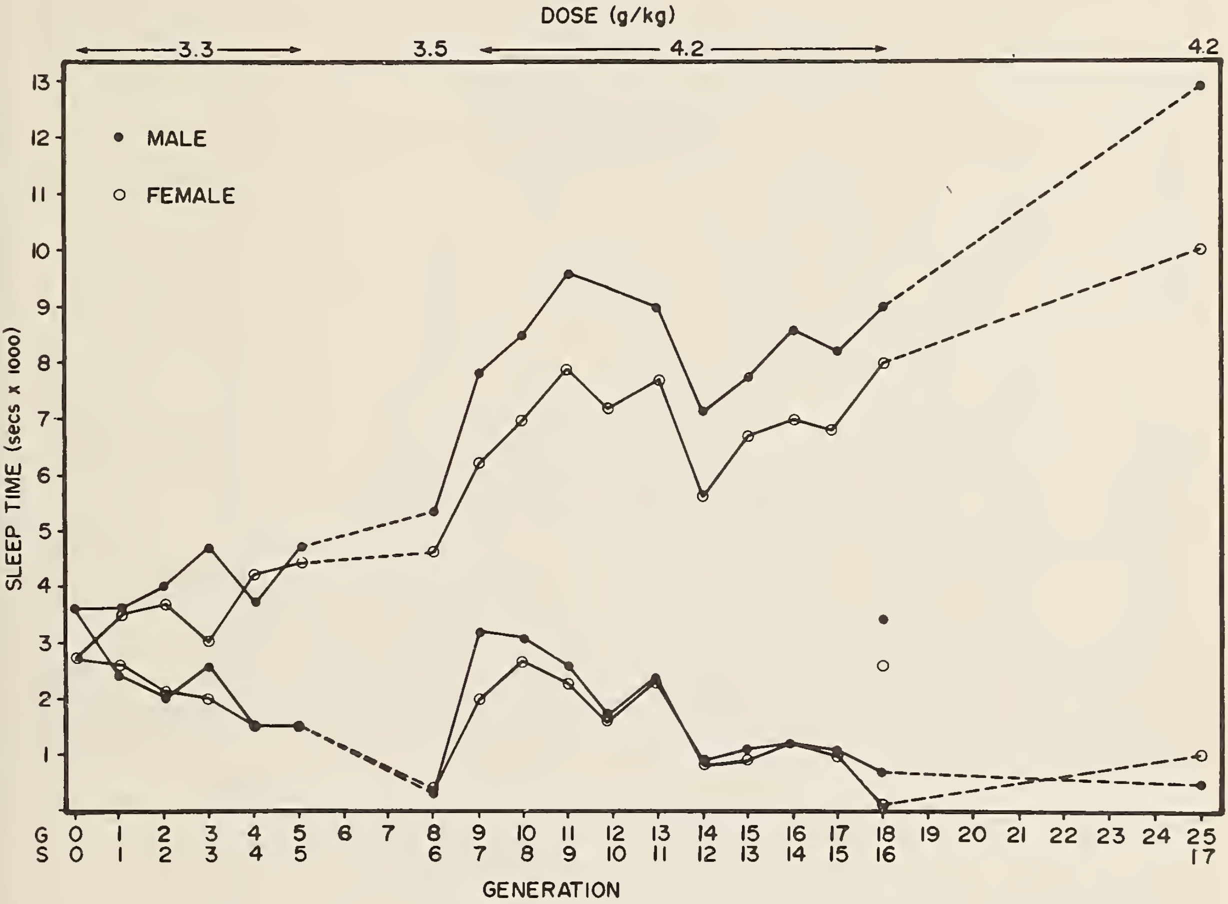
Figure 1. Distributions of Sleep Time Scores for the Foundation Stock and the First Five Selected Generations



The top distribution shows sleep time scores of the genetically diverse population of mice from which the selected lines were derived. The other distributions illustrate divergence of the lines due to upward (open bars) and downward (shaded bars) selective breeding over five generations of selection.

Reproduced with the publisher's permission from McClearn (1981). Copyright 1981 by Academic Press.

Figure 2. Means of Sleep Time Scores by Sex and by Generation for the LS and SS Lines



The top two functions represent the LS line, and the bottom two represent the SS. Data points at S_{16} indicate mean values for male and female HS mice tested contemporaneously with S_{16} LS and SS animals. Dashed lines indicate suspension of selection.

response to selection. Figure 4 shows, however, that no systematic divergence between lines occurred for fall time from S_0 through S_5. This outcome might reflect an absence of common loci for the two phenotypes or might simply be due to low reliability of the fall time measure. If the former alternative is correct, the results would demonstrate the complexity of the domain of "first-dose sensitivity" and suggest the possibility of genetic dissection of the domain.

On the basis of these results, the decision was made to cease fall time assessment and to use the simpler restraint time procedure to define the beginning of the sleep time period. Figure 5 shows that divergence has occurred for this measure, with the SS animals

Figure 3. Frequency Distributions of Sleep Time Scores of S_{17} LS and SS Mice

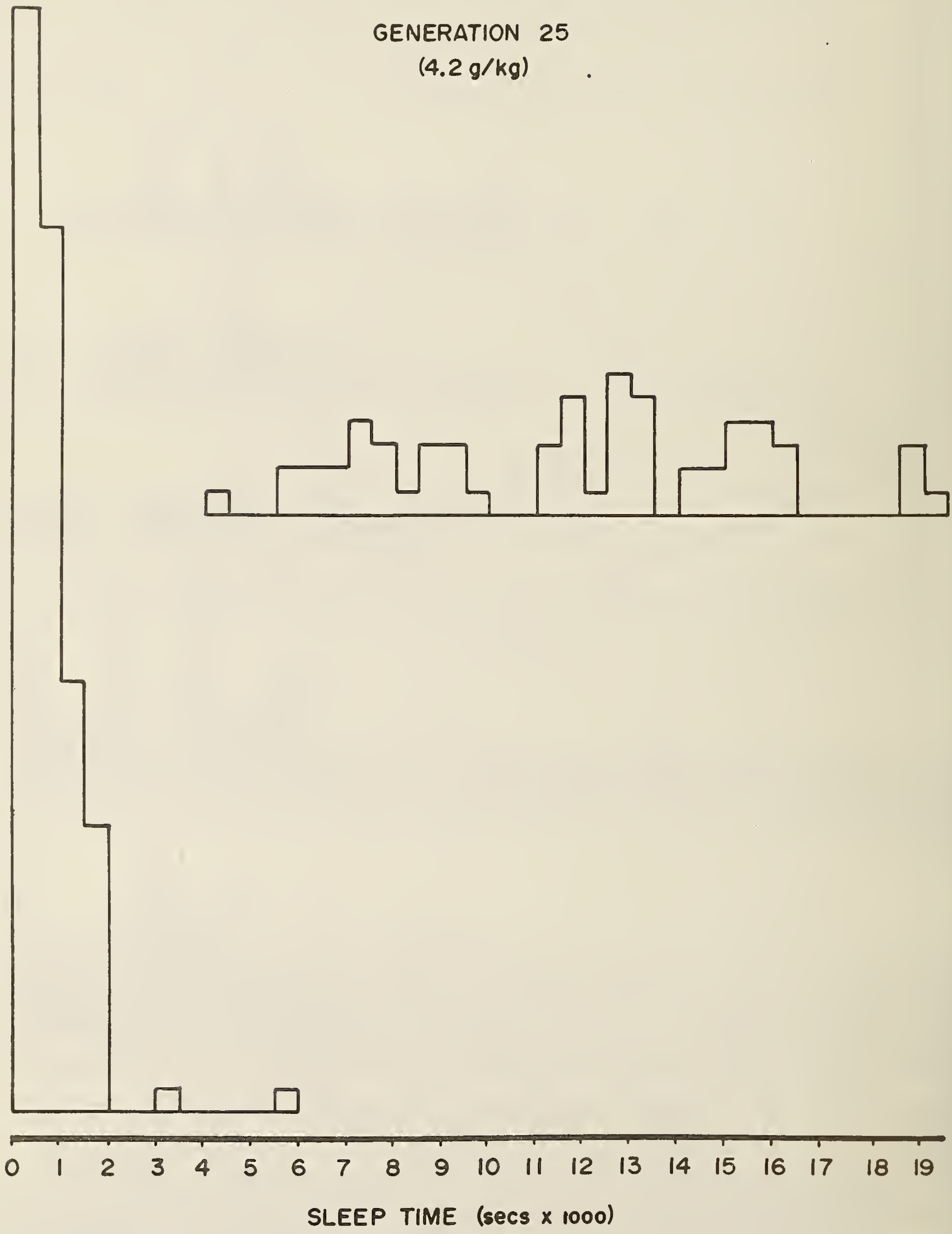
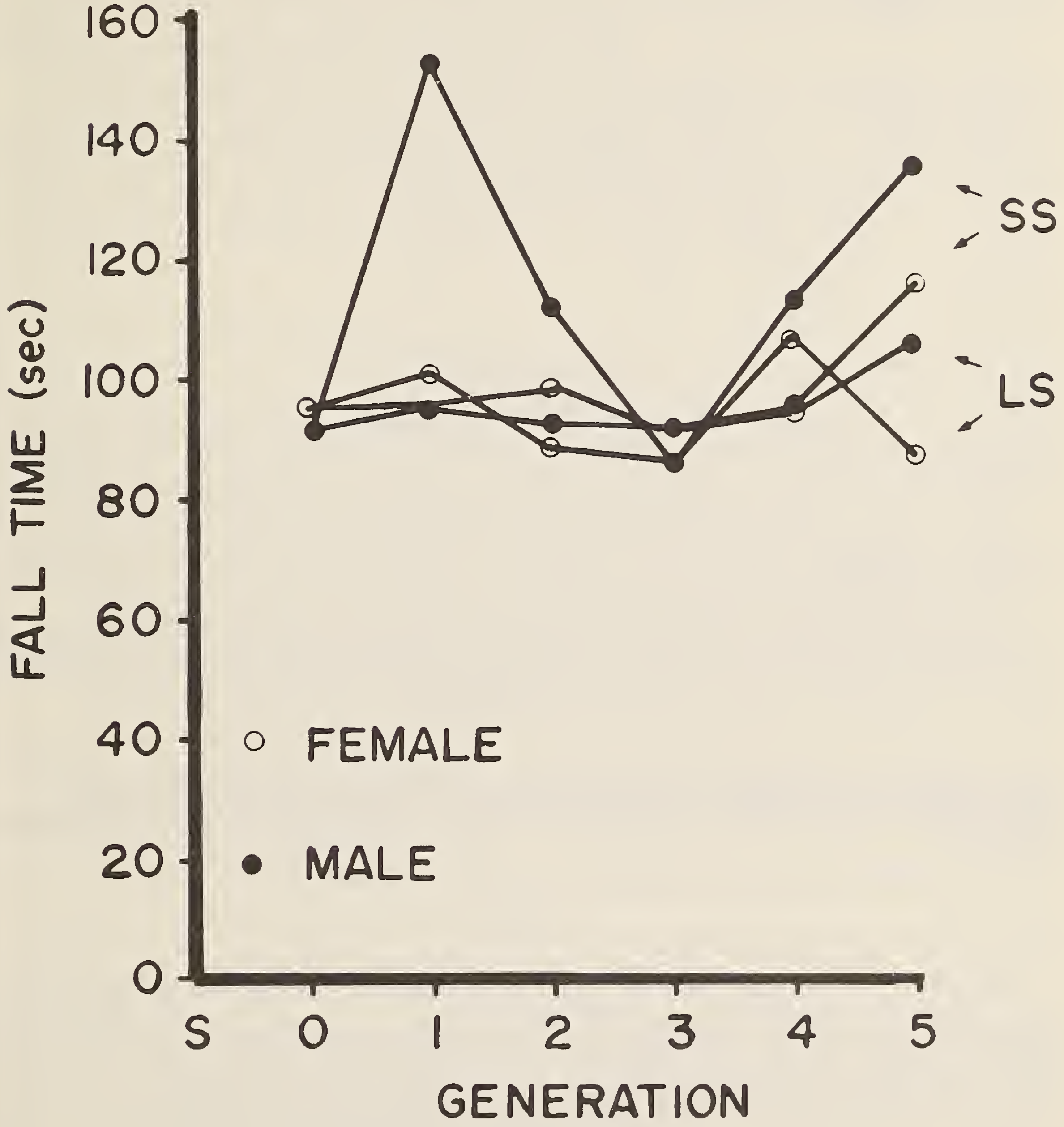
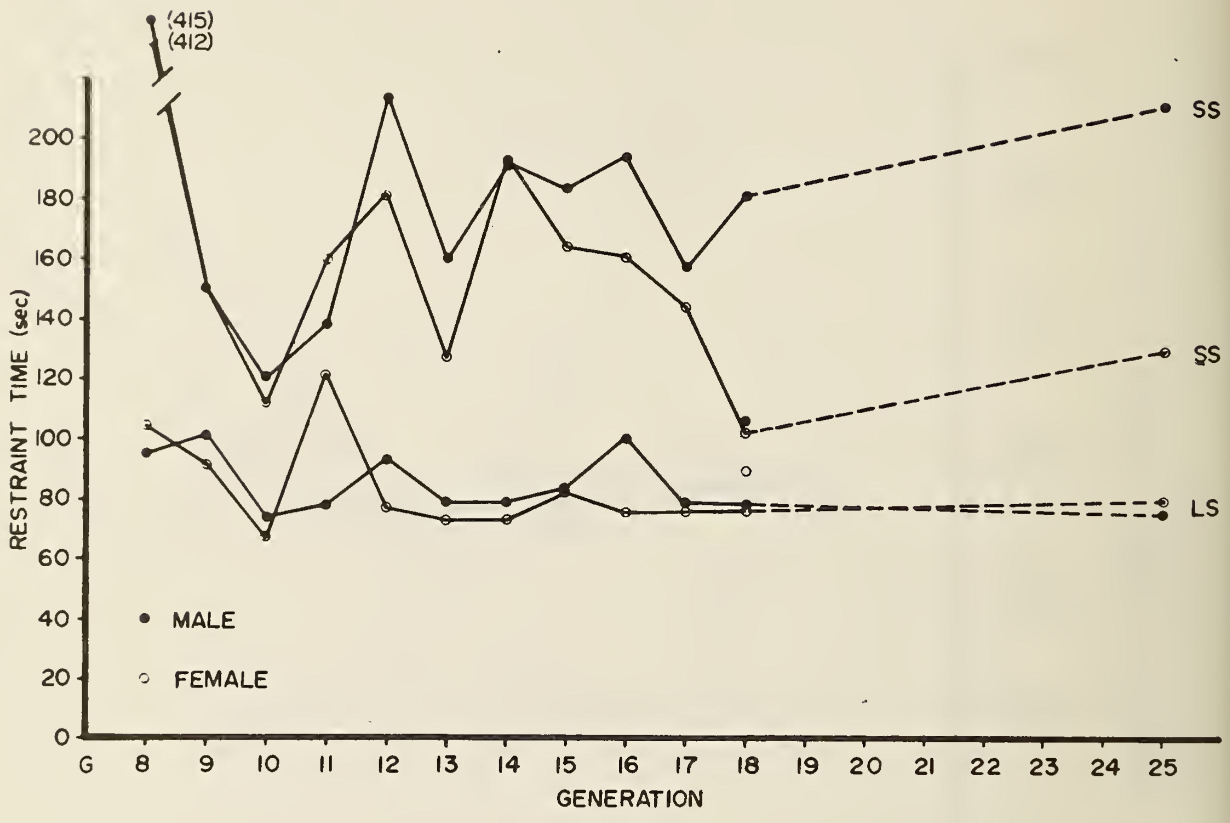


Figure 4. Means of Fall Time Scores by Sex and by Line from S_0 Through S_5



requiring longer restraint before the righting response is lost. The locations of the restraint time means for male and female HS mice tested contemporaneously with G_{18} (S_{16}) are also shown in the figure. The very high restraint times for the SS line in G_8 (S_6) may be due to the increase in dosage from 3.3 g/kg to 3.5 g/kg in that generation. There is no obvious explanation for the sex differences in the SS line in G_{18} (S_{16}) and G_{25} (S_{17}).

Figure 5. Means of Restraint Time Scores by Sex and by Line from G_8 (S_6) Through G_{25} (S_{17})



Data points at G_{18} indicate mean values for male and female HS mice tested contemporaneously with G_{18} LS and SS animals. Dashed lines indicate suspension of selection.

Whether a correlated fall time response to selection would have begun in S_6 or S_7 or whether fall time scores would have remained the same in the two lines cannot, of course, be determined without further research. If fall time and restraint time are, in fact, genetically unrelated, the complexity of first-dose sensitivity would be emphasized.

Conclusion

Although the inbreeding that accompanied the fertility problems in G_6 and G_7 is regrettable, the continued response to selection reveals that additive genetic variance remained after the "bottleneck." This outcome is to be expected, of course, because many consecutive generations of inbreeding are required for nearly all loci to become fixed (Falconer 1960). Nevertheless, the inbreeding that did occur prompts some caution in interpreting mean line differences in other characteristics as being part of the causal mechanisms that determine the sleep time difference (McClearn 1979).

A breeding program of continued selection after crossing each line separately with a genetically heterogeneous stock is contemplated. This procedure would assure restoration and maintenance of the desired heterogeneity at all loci not relevant to sleep time.

References

Falconer, D.S. *Introduction to Quantitative Genetics.* New York: Ronald Press, 1960.

Kakihana, R.; Brown, D.R.; McClearn, G.E.; and Tabershaw, I.R. Brain sensitivity to alcohol in inbred mouse strains. *Science,* 154:1574-1575, 1966.

McClearn, G.E. Genetic differences in the effect of alcohol upon behavior of mice. In: Havard, J.D.J., ed. *Proceedings of the Third International Conference on Alcohol and Road Traffic.* London: British Medical Association, 1962. pp. 153-155.

McClearn, G.E. Influence of genetic variables on means, variances, and covariances in behavioral responses to toxicological and pharmacological substances. *J Toxicol Environ Health,* 5:145-156, 1979.

McClearn, G.E. Evolution and genetic variability. In: E.S. Gollin, ed. *Developmental Plasticity: Behavioral and Biological Aspects of Variations in Development.* New York: Academic Press, 1981.

McClearn, G.E., and Kakihana, R. Selective breeding for ethanol sensitivity in mice. (Abstr.) *Behav Genet,* 3:409-410, 1973.

McClearn, G.E., and Rodgers, D.A. Differences in alcohol preference among inbred strains of mice. *Q J Stud Alcohol,* 20:691-695, 1959.

McClearn, G.E.; Wilson, J.R.; and Meredith, W. The use of isogenic and heterogenic mouse stocks in behavioral research. In: Lindzey, G., and Thiessen, D.D., eds. *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype.* New York: Appleton-Century-Crofts, 1970. pp. 3-22.

A Review of Research Using Short-Sleep and Long-Sleep Mice*

Allan C. Collins

The genetics and development of the long-sleep (LS) and short-sleep (SS) lines of mice have been discussed by McClearn and Kakihana. This chapter attempts to summarize and critique the published studies that have focused on the use of these selected lines as research tools to test relevant hypotheses concerning the actions of ethyl alcohol.

Although the development of the LS and SS lines was initiated in 1963, significant pharmacological research was not begun until just a few years ago. Studies that will be discussed here utilized animals from the 14th to the 25th generation, i.e., the 12th to the 17th selected generation. Thus, there is no early history of the divergence of any of the biochemical and physiological parameters that appear to differentiate the LS and SS mice.

Selective breeding for differential sensitivity to the hypnotic effects of ethanol should, at least theoretically, have selected for all of the genes that would influence the duration of hypnosis induced by large doses of ethanol. On an a priori basis, it would be expected that LS mice would have a greater central nervous system (CNS) sensitivity to the depressant effects of ethanol coupled with a relatively slow rate of ethanol metabolism. The SS mice, on the other hand, would be expected to demonstrate a lesser CNS sensitivity to ethanol and a faster rate of ethanol elimination. Several studies have dealt with this hypothesis. Heston et al. (1974) measured the rates of ethanol disappearance in the LS and SS lines following the intraperitoneal injection of ethanol. Somewhat surprisingly, the rates of ethanol disappearance seemed to be identical in the two lines. More recently, Kakihana (1977) replicated this result. Heston and coworkers also measured the activities of hepatic alcohol and aldehyde dehydrogenases in the two lines of mice. Their finding of no differences is consistent with the identical rates of in vivo elimination. These experiments provide evidence to support the notion that the LS and SS mice differ only in CNS sensitivity to ethyl alcohol and do not differ in their metabolism of this agent.

---

*This review was written while the author was supported by a Research Scientist Development Award (AA-00029) from the National Institute on Alcohol Abuse and Alcoholism.

Only one study has assessed the activities of the hepatic microsomal ethanol-oxidizing system (MEOS) in the LS and SS mice. French et al. (1979) measured both basal MEOS activity and activity induced by 3-methyl cholanthrene in the two lines. No difference was observed in basal activity of this system, but a difference in inducibility was noted. The SS mice appear to be inducible, or responsive, while the LS mice are noninducible, or nonresponsive. The lines therefore have the potential for different activities of the MEOS system after chronic ethanol treatment because of a differential induction of MEOS activity. Whether ethanol induces MEOS activity in the LS and SS mice has not been examined, however.

No adequate explanation has been presented as to why selective breeding failed to produce a differential rate of ethanol elimination in the LS and SS lines. However, this result has been advantageous to those researchers attempting to provide neurochemical explanations for differential behavioral responses to alcohol in the two lines, because behavioral differences were not confounded by differences in ethanol metabolism.

While there seems to be no significant difference between the lines with respect to rate of ethanol metabolism, there appear to be subtle differences between them in distribution of ethanol. An early study by Heston et al. (1974) found that LS mice had a slightly higher blood ethanol concentration than did SS mice at all time points tested. This observation has been replicated in a number of unpublished studies carried out in the author's laboratory. A possible explanation for such a phenomenon is a differential volume distribution for ethanol in the two lines. The LS mice appear to have a smaller volume of distribution than the SS mice.

Pharmacological Effects of Alcohol and Other Drugs

Further support for the contention that the LS and SS mice differ in CNS sensitivity to ethanol comes from a series of experiments reported by Erwin et al. (1976). In these experiments, "waking blood alcohol" (ethanol concentration in the blood measured at the time of regaining the righting reflex) was measured in the LS and SS mice when they regained the righting reflex following the intraperitoneal injection of a 4.1 g/kg ethanol dose. Waking blood alcohol concentration was 1.65 times greater in the SS mice than in the LS mice. Erwin and coworkers also determined that the ED_{50} value for loss of the righting reflex in the two lines after ethanol injection was 3.64 g/kg for the SS line and 1.65 g/kg for the LS line. Loss of the righting reflex occurs before substantial metabolism has occurred. Taken together, these two experiments clearly suggest that the LS and SS mice were selected primarily for differential CNS sensitivity to ethanol.

Erwin et al. (1976) also constructed dose-response curves for loss of the righting reflex for methyl, butyl, and t-butyl alcohol, as well as for pentobarbital and diethyl ether. The dose-response curves obtained for the types of alcohol were parallel to those obtained for ethanol, suggesting a similarity in mechanism of action among the three types of alcohol. The LS and SS mice differed in sensitivity to these other alcohol types in a fashion similar to that observed for ethanol. The lines did not manifest a differential sensitivity to pentobarbital or to diethyl ether. This result suggests that alcohol exerts its CNS depressant effects via mechanisms different from those that explain the actions of barbiturates and ether. The observation that sleep time values obtained after administration of pentobarbital, chloral hydrate, trichloroethanol, or paraldehyde are not different in the two lines further supports this specificity of action hypothesis.

A subsequent study by Sanders et al. (1978) raises some questions regarding the specificity of ethanol's action. These investigators determined sleep times following trichloroethanol, paraldehyde, and pentobarbital in mice from the 19th, 20th, and 21st generations of the LS and SS lines. Significantly longer sleep times were obtained in the LS line for trichloroethanol and paraldehyde, while no difference was detected for pentobarbital. It should be noted that the differences obtained were much smaller than those observed for alcohol in animals obtained from the same generations. A possible interpretation of these results is that, with continued selection, genes or gene products that influence the hypnotic effects of trichloroethanol and paraldehyde share at least partial identity with those genes or gene products that mediate the actions of alcohol. This conclusion should be viewed cautiously, however, because differences in volume of distribution, metabolism, or clearance could conceivably have caused the differences in sleep time observed in this study.

Siemens and Chan (1976) measured sleep times in the LS and SS lines following alcohol or pentobarbital injection. The usual sleep time differences were observed for alcohol, while pentobarbital produced a significantly longer sleep time in SS mice than in LS mice. Waking brain pentobarbital levels were determined and, although the value obtained in SS mice was slightly lower than that seen in the LS line, the variance in the measure was large; however, statistical significance was not attained. Seimens and Chan also determined the rates of pentobarbital disappearance in the two lines. Essentially identical rates were seen, but the apparent volume of distribution for pentobarbital was greater in the LS line, a situation which is opposite to that seen for ethanol. Siemens and Chan suggested that the differences in pentobarbital sleep time observed in their study might have arisen as a result of the difference in apparent volume of distributrion.

Data currently available suggest at least some specificity in differential response to sedative-hypnotic drugs between the LS and SS mice. Only when studies concerned with specificity of drug response involve a determination of relative metabolism and distribution can any unequivocal statements be made regarding the commonality, or lack thereof, of mechanisms between ethanol and other sedative-hypnotic or anesthetic agents. Results of those studies that have investigated these parameters lead to the conclusion that the LS and SS mice differ in CNS sensitivity to alcohol, and alcohol only.

Other Alcohol-Related Behaviors

Most of the behavioral studies utilizing the LS and SS mice have focused on sleep time. However, the two lines do manifest a differential sensitivity to other actions of alcohol. Sanders (1976) demonstrated that the SS mice are more affected by low doses of alcohol than are the LS mice when behavioral activation in an open-field arena is measured. Kakihana (1977) determined the effect of a 2 g/kg dose of ethanol on rectal temperature. The SS line showed a mean decrease of 1.1°C which lasted for about 2 hours, while the LS line showed a decrease of 2.6°C which lasted for 6 hours. Kakihana suggested that the lesser decrease in the SS mice may have been related to the greater degree of behavioral activation observed in this line.

Fuller and Church (1977) studied voluntary alcohol consumption in LS and SS mice. Their data suggest that the SS mice may consume more ethanol than the LS mice if the ethanol solution is sweetened with 3 percent glucose and 0.16 percent sodium saccharin. We have not detected any differences between the lines if unsweetened 10 percent ethanol and water are presented as the choice solutions (unpublished results). Both lines totally avoid the ethanol solution. Thus, the SS mice may prefer ethanol more than the LS mice only when a sweetener is added.

Goldstein and Kakihana (1975) examined severity of alcohol withdrawal following 3 days of treatment using Goldstein's classical pyrazole-alcohol vapor technique. The SS mice exhibited a much more severe withdrawal syndrome than did the LS mice. The correlation between severity of withdrawal and alcohol sleep time in contemporaneous heterogeneous stock (HS) mice was also determined. No correlation was observed, and the conclusion was drawn that a lesser CNS sensitivity to alcohol does not result in greater withdrawal severity as the LS-SS data suggest. However, it should be noted that wide variances in alcohol metabolism exist among HS mice. Goldstein and Kakihana measured only sleep time, which is influenced by both CNS sensitivity and metabolism rate, rather than directly measuring CNS sensitivity to alcohol. Thus, it may be that a correlation between initial sensitivity to alcohol and withdrawal severity following chronic treatment does exist. Obviously this possibility needs to be examined more closely.

Neurotransmitters

GABA

Chan (1976) measured regional GABA levels in control and ethanol-treated (4 g/kg) LS and SS mice. Ethanol caused an increase in GABA levels in the cerebrum, cerebellum, and thalamus-hypothalamus of LS mice 30 minutes after injection. No signficant effect was seen in the medulla. Significant increases in GABA were seen in all of these areas in SS mice at 30 minutes. No signficant effect was detected in either line 90 minutes after alcohol administration. Control levels did not differ significantly between the lines in any of the brain regions studied. Chan concluded that these data suggest no correlation between brain GABA and CNS sensitivity to ethanol.

Norepinephrine and Dopamine

Collins et al. (1976) measured whole brain norepinephrine (NE) and dopamine (DA) in the LS and SS lines, as well as the effect of a 4.1 g/kg ethanol dose on turnover rates of these neurotransmitter amines. Turnover rates were assessed by treating the animals with alpha-methyl-p-tyrosine and assessing the effects of ethanol on the rate of decline in brain NE and DA content. Control concentrations of both NE and DA were significantly greater in the SS line. Perhaps more important, ethanol had a marked effect on DA but not NE turnover rate. Ethanol induced a 69 percent reduction in DA turnover in the LS line and a 35 percent reduction in SS mice. These data suggest a differential effect of ethanol on DA-containing neurons in the two lines. Because synthesis inhibition was used to assess turnover rate, our data imply that ethanol either inhibits DA release or increases its uptake. The findings of other investigators suggest an inhibition of release as the most plausible explanation for ethanol's effect on DA turnover.

Dudek and Fanelli (1980) added further support to the notion that dopamine release is involved in ethanol's actions. He examined the effects of high doses of gammabutyrolacetone (GBL) on loss of the righting reflex in the two lines. GBL, which inhibits impulse transmission in dopaminergic systems, produced a longer loss of the righting response in LS than in SS mice.

Church et al. (1976) examined the effects of intraventricular injections of salsolinol, the dopamine-acetaldehyde condensation product, on open-field activity in LS and SS mice. Control SS mice were found to be more active in the arena than were LS mice, and salsolinol decreased activity in both lines. The LS line was more sensitive to this effect. These investigators also examined the effects of high doses of salsolinol on duration of loss of the righting reflex. The LS mice lost the righting reflex for a longer time than did the SS mice.

Taken together, these data suggest a role for dopamine or for a metabolite such as salsolinol in the depressant effects of ethanol.

Alcohol-Corticosteroid Interactions

Two studies by Kakihana (1976, 1977) explored the effects of ethanol on adrenocortical response. The adrenal glands of SS mice are 20-30 percent heavier than those of LS mice, and a mild stress, such as saline injection, causes a greater release of plasma corticosteroids in SS mice. Thus, although injection of ethanol causes an equal rise in plasma corticosteroids in the two lines, it appears that the net influence of ethanol on corticosteroid release is greater in the LS line.

The mild stress-induced difference in corticosteroid response (with SS being more affected than LS) may make an important contribution to the difference in "emotionality" between the two lines. Although the phenomenon has not been studied adequately, all who have worked with the LS and SS mice can attest to the fact that the SS mice are much more active and aggressive than the docile LS animals.

Seizure Threshold

The apparent difference in excitability was the stimulus for a series of experiments which investigated the response of LS and SS mice to the convulsant flurothyl. Greer and Alpern (1977) noted that SS mice had significantly shorter latencies to myoclonus but significantly longer latencies to the onset of clonus than did LS mice. Because myoclonus and clonus did not correlate positively with one another in the two lines, these investigators suggested that the two convulsive components might be mediated by different substrates. Data obtained with LS and SS mice pretreated with apomorphine and haloperidol, which affect dopaminergic systems, suggest that dopaminergic systems influence onset of myclonus but not clonus; data obtained with mice pretreated with pilocarpine and scopolamine, which influence cholinergic transmission, suggest that cholinergic systems influence onset of clonus. An additional study utilizing bicuculline, a GABA receptor blocker, suggested that clonus is controlled by GABA pathways as well as cholinergic pathways. An inference drawn from these studies is that SS mice have a more active dopaminergic system than do LS mice, a result which agrees with the study by Collins et al. (1976), whereas SS mice may have a less active cholinergic system than do LS mice.

Another study by Alpern and Greer (1977) added further credence to the notion that dopaminergic systems influence myoclonus in these two lines of mice and that this system undergoes a maturational change in the SS animals. Onset of flurothyl-induced myoclonus was delayed by amphetamines in young (30-day-old) SS mice, whereas amphetamine decreased the latency to onset of myoclonus in mature (250-day-old) SS mice. The LS mice, both young and mature, responded to amphetamine in a fashion similar to the mature SS mice; i.e., a decrease in latency. Because amphetamine affects both noradrenergic and dopaminergic systems, Alpern and Greer assessed the effects of apomorphine (a dopamine receptor agonist) and clonidine (an alpha noradrenergic antagonist) on latency to myoclonus in young and mature LS and SS mice. Apomorphine pretreatment acted like amphetamine pretreatment; i.e., it increased latency to onset of myoclonus in young SS mice, while it decreased latency in mature SS mice and in both young and mature LS mice. These data suggest a developmental change in sensitivity or function of the dopaminergic system in SS mice. No such change was obvious for noradrenergic systems, because clonidine decreased latency to myoclonus in both young and mature LS and SS mice. Alpern has been pursuing these studies in connection with his investigation of the possibility that SS mice may serve as an animal model for hyperkinesis.

These data add support to the neurochemical observation that alcohol differentially affects dopamine turnover in the LS and SS lines. However, a direct comparison between studies may be unwise because the neurochemical studies were carried out with animals that were 51-83 days old. Until more information has been obtained concerning the possible developmental change in dopaminergic systems, studies examining the role of dopamine in ethanol's effects probably should take very special pains to control for age. In addition, more recent efforts, carried out in our laboratory, using more modern assay methods, have failed to replicate the earlier observation of a difference in whole-brain norepinephrine and dopamine content. These studies did not examine the effects of ethanol on catecholamine turnover.

A recent study by Sanders and Sharpless (1978) raised the question as to whether the difference in sensitivity to flurothyl-induced convulsions is related to the mechanisms by which alcohol elicits its behavioral effects. These investigators assessed the effects of low doses of ethanol or pentobarbital on seizure latencies. Ethanol and pentobarbital increased seizure latencies in both populations, and there was no difference between the lines in the effect of these drugs. The investigators suggested that the lack of difference in response to ethanol in the two lines indicates that the genetic mechanisms that determine sensitivity to ethanol's anticonvulsant action are not identical with those that determine sensitivity to its depressant action. This notion is consistent with the suggestion of Goldstein and Kakihana (1975) that withdrawal severity is not directly related to sensitivity to the acute depressant effects of ethanol. In fact, SS mice may show a more severe alcohol withdrawal syndrome because of their more excitable nervous system and not because of a lesser initial sensitivity to alcohol's depressant effects.

Conclusion

The use of the LS and SS mice in testing hypotheses relevant to alcohol research is clearly in its infancy. All of the studies carried out thus far have consisted of line comparisons. Results of these studies clearly show that lines do not differ in ethanol metabolism but that they do differ in CNS sensitivity to ethanol. Available data suggest a role for dopamine in the differential sensitivity to ethanol, but procedural differences among the studies suggest that this notion should be viewed cautiously. Future experiments designed to test the hypothesis that dopamine is involved in ethanol's depressant effects should keep in mind that any differences detected may be unrelated to the direct effects of ethanol. A more careful genetic analysis using F_1 and F_2 generations derived by crossing the LS and SS lines could be of value in resolving this issue and could be used for testing other relevant hypotheses.

References

Alpern, H.P., and Greer, C.A. A dopaminergic basis for the effects of amphetamine on a mouse "preadolescent hyperkinetic" model. *Life Sci*, 21:93-98, 1977.

Chan, A.W.K. Gamma aminobutyric acid in different strains of mice: Effect of ethanol. *Life Sci*, 19:597-604, 1976.

Church, A.C.; Fuller, J.L.; and Dudek, B.C. Salsolinol differentially affects mice selected for sensitivity to alcohol. *Psychopharmacology*, 47:49-52, 1976.

Collins, A.C.; Lebsack, M.E.; and Yeager, T.N. Mechanisms that underlie sex-linked and genotypically determined differences in the depressant actions of alcohol. *Ann NY Acad Sci*, 273:303-316, 1976.

Dudek, B.C., and Fanelli, R.J. Effects of gammabutyrolactone, amphetamine, and haloperidol in mice differing in sensitivity to alcohol. *Psychopharmacology*, 68:68-97, 1980.

Erwin, V.G.; Heston, W.D.W.; McClearn, G.E.; and Dietrich, R.A. Effect of hypnotics on mice genetically selected for sensitivity to alcohol. *Pharmacol Biochem Behav*, 4:679-683, 1976.

French, T.A.: Atkinson, N.; Petersen, D.R.; and Chung, L.W.K. Differential induction of hepatic microsomal ethanol and drug metabolism by 3-methylcholanthrene in LS and SS mice. *J Pharmacol Exp Ther*, 209:404-410, 1979.

Fuller, J.L., and Church, A.C. Voluntary ethanol consumption by mice selected for differing sleep times following acute ethanol administration. (Abstr.) *Behav Genet*, 7:59, 1977.

Goldstein, D.B., and Kakihana, R. Alcohol withdrawal reactions in mouse strains selectively bred for long or short sleep times. *Life Sci*, 17:981-986, 1975.

Greer, C.A., and Alpern, H.P. Mediation of myoclonic seizures by dopamine and clonic seizures by acetylcholine and GABA. *Life Sci*, 21:385-392, 1977.

Heston, W.D.W.; Erwin, V.G.; Anderson, S.M.; and Robbins, H. A comparison of the effects of alcohol on mice selectively bred for differences in ethanol sleep-time. *Life Sci*, 14:365-370, 1974.

Kakihana, R. Adrenocortical functions in mice selectively bred for different sensitivity to ethanol. *Life Sci*, 18:1131-1138, 1976.

Kakihana, R. Endocrine and autonomic studies in mice selectively bred for different sensitivity to ethanol. In: Gross, M., ed. *Alcohol Intoxication and Withdrawal.* Vol. IIIA. (*Vol. 85a of Advances in Experimental Medicine and Biology.*) New York: Plenum Press, 1977. pp. 83-95.

Sanders, B. Sensitivity to low doses of ethanol and pentobarbital in mice selected for sensitivity to hypnotic doses of ethanol. *J Comp Physiol Psychol*, 19:394-398, 1976.

Sanders, B., and Sharpless, S.K. Dissociation between the anticonvulsant actions of alcohol and its depressant action in mice of different genotypes. *Life Sci*, 23:2593-2600, 1978.

Sanders, B.; Sharpless, S.K.; Collins, A.C.; McClearn, G.E.; and Flanagan, C. Activating and anesthetic effects of general depressants. *Psychopharmacology*, 56:185-189, 1978.

Siemens, A.J., and Chan, A.W.K. Differential effects of pentobarbital and ethanol in mice. *Life Sci*, 19:581-589, 1976.

Indiana Selection Studies on Alcohol-Related Behaviors*

T.-K. Li, L. Lumeng, W.J. McBride, and M.B. Waller

Alcoholism has been defined as a disease of pathological dependency on ethanol (National Council on Alcoholism 1972). It is a behavioral disorder in which the excessive and uncontrolled drinking of alcoholic beverages leads to tolerance, dependence, organ damage, and social opprobrium. Although it is evident that the causative agent of alcoholism is ethanol, researchers do not understand the basis of individual variation in the host's response to this agent. In other words, we do not yet know why some individuals are more susceptible to alcoholism than others and why some alcoholics are more susceptible to the medical complications of alcoholism than are other alcoholics.

At this point, alcoholism remains a disorder unique to humans; it has not been observed to occur spontaneously nor has it been produced experimentally in other animal species. This circumstance has seriously hampered efforts to discern the underlying biologic determinants of the disorder. However, in recent years increasingly convincing evidence has been gathered that predisposition to alcoholism is significantly influenced by inherited factors (Cadoret and Gath 1978; Goodwin et al. 1973). As an experimental approach, therefore, a logical beginning would be to look for genetic differences in susceptibility to certain of the key pathophysiologic processes in the natural history of the disorder, to elucidate the underlying biochemistry and physiology of such differences, and to discern their contribution and interactions in the production of the alcoholic state.

---

*This work was supported by U.S. Public Health Service Grant AA-03243. The skillful technical assistance of Marilynn McKee and Martha Clark is gratefully acknowledged.

Some of these key pathophysiologic processes are shown in table 1, which also identifies the processes for which a genetic influence has either been discovered or suggested. The data for humans derive principally from studies of twins and different racial groups (Blass and Gibson 1977; Partanen et al. 1966; Vesell et al. 1971; Wolff 1972). Those for experimental animals come almost entirely from the studies being reported in this conference monograph.

Table 1. Aspects of Alcoholism Exhibiting Genetic Influence

Aspect	Human	Experimental Animals
Drinking behavior	X	X
Innate tolerance or sensitivity	X	X
Ethanol metabolic rate	X	
Acquired (metabolic) tolerance		X
Physical dependence		X
Susceptibility to medical complications	?	

It has been the authors' viewpoint that, among the processes listed in table 1, drinking behavior is operationally the first and most important variable to control experimentally, if the objective is to develop an animal model of alcoholism. The reason is that all other necessary conditions in the pathogenesis of alcoholism, whatever they may be, are contingent upon the acquisition of ethanol through drinking. Moreover, knowledge about factors that contribute to aberrancy in drinking behavior is fundamental to our understanding of this disorder. Therefore, we have chosen rats, selectively bred for high alcohol preference, as the primary experimental model. It is our hypothesis that such animals can be developed into a suitable model of alcoholism according to the criteria shown in table 2. These criteria are similar to those previously suggested by Cicero and Smithloff (1973) and by Lester and Freed (1973), and emphasize the importance of voluntary oral consumption, independent of the caloric and gustatory properties of ethanol. This chapter reviews and updates our studies and efforts in this direction.

Table 2. Animal Model of Alcoholism: Suggested Criteria

1. Ethanol should be self-administered orally.
2. Amount should elevate blood alcohol concentration and approach or exceed limit of metabolic capacity.
3. Consumption should be for the CNS pharmacologic effects of ethanol (not because of caloric value, taste, or smell).
4. Ethanol should be positively reinforcing.
5. Consumption should ultimately result in:
    (a) repeated bouts of intoxication,
    (b) tolerance,
    (c) physical dependence.

The Development of Alcohol-Preferring and Alcohol-Nonpreferring Lines of Rats by Selective Breeding

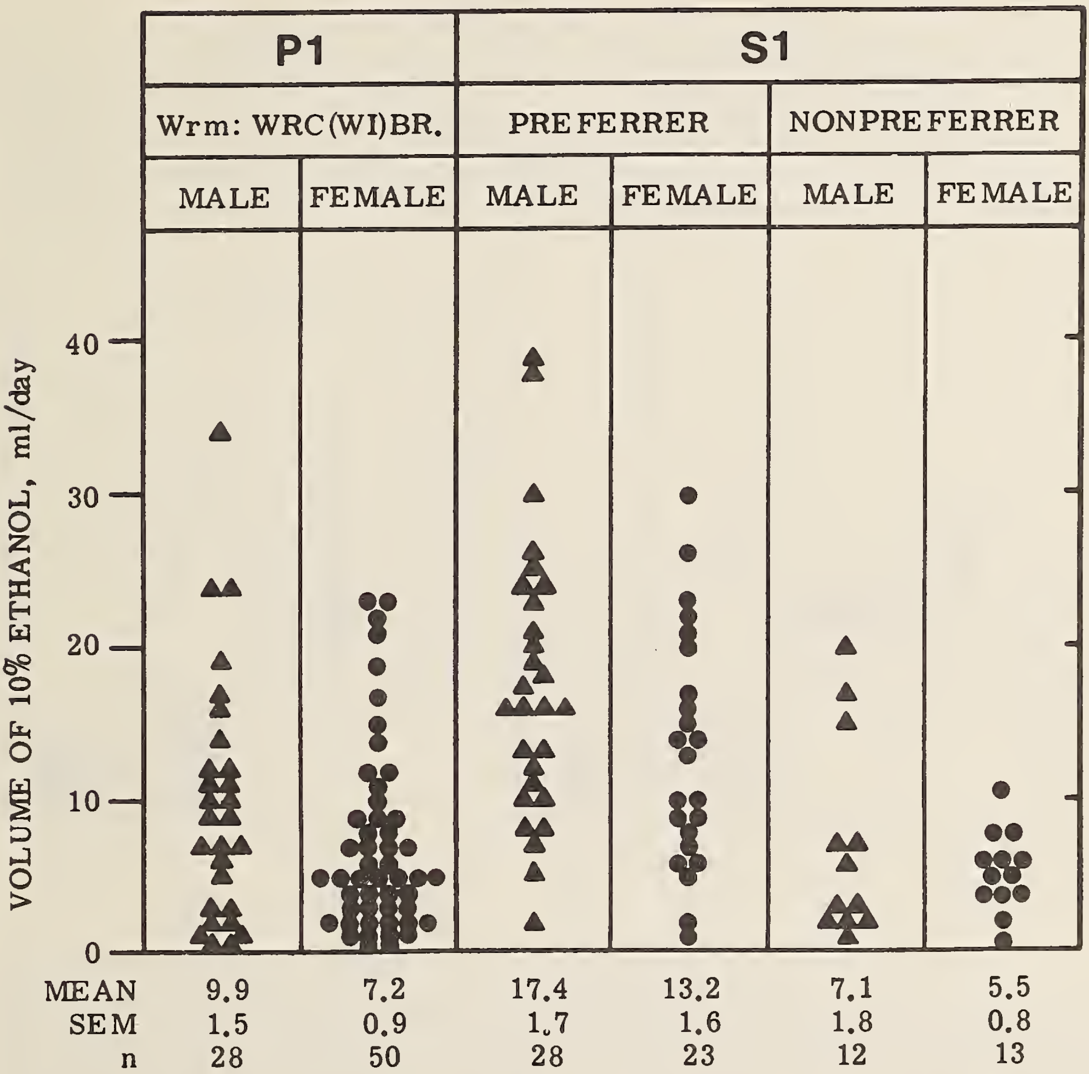
Although most animals find the drinking of solutions containing moderate to large amounts of ethanol aversive, rats and inbred strains of mice innately exhibit large individual variations with regard to alcohol preference. In common stock rats, this natural variation may be quite extreme. While most rats consume less than 3 g ethanol/kg body weight per day, a small percentage may be found to consume 6-8 grams or more. Through inbreeding from common stock animals (Mardones 1960) and selective breeding from heterogeneous stock animals (Eriksson 1971) alcohol-preferring and alcohol-nonpreferring lines have been raised.

The foundation stock from which the alcohol-preferring (P) and the alcohol-nonpreferring (NP) lines are derived is a randomly bred, closed colony of Wistar rats (Wrm:WRC(WI)BR) from the Walter Reed Army Institute of Research, Washington, D.C. The voluntary alcohol drinking behavior of these rats was assessed by a two-bottle preference test, the method now routinely employed for selection in our laboratory (Lumeng et al. 1977). In this procedure, the rats are housed individually after the onset of puberty and are given 10 percent ethanol as the sole source of fluid for the first 4 days. Thereafter, the animals are given access to both 10 percent ethanol and water in graduated Richter tubes for 3 weeks. The locations of the tubes are changed on a random basis each day after the volumes of water and ethanol solution consumed have been recorded. Food is provided ad libitum at all times. The volume of 10 percent ethanol consumed daily during the last 2 weeks of testing is averaged to give the consumption score of the individual animal. As is evident from figure 1, considerable heterogeneity in voluntary ethanol consumption was found in the stock animals. From 78 animals tested for alcohol preference in this manner, a single pair with high preference were mated and a single pair with low preference were mated in order to start the P and NP lines, respectively. Their offspring (S_1 generation) exhibited a similar degree of heterogeneity with respect to alcohol preference (figure 1).

Selective breeding was continued by the mating of males and females with the highest ethanol preference in the P line and by the mating of males and females with the lowest preference in the NP line of each succeeding generation. Segregation between lines began to appear in the S_2 generation (figure 2), and as reported recently, the voluntary ethanol consumption of the P and NP animals in the S_8 generation was 5.2 ± 2.2 (mean ± S.D.) and 1.7 ± 0.9 g/kg body weight/day, respectively (Lumeng et al. 1977). By comparison, the amount of ethanol consumed by the P and NP animals in the S_{10} and S_{11} generations (figure 2) was 6.3 ± 1.7 and 1.0 ± 0.7 g/kg/day, respectively. Thus selection pressure has been maintained, and increased homogeneity within lines has been accomplished. These consumption scores are comparable with several generations of the selectively bred AA and ANA rats developed by Eriksson from heterogeneous stock animals (Ahtee 1972; Eriksson 1972; Eriksson and Närhi 1972; Rusi et al. 1977), and the difference between the P and NP lines is greater than that between the inbred lines of alcohol-preferring and alcohol-nonpreferring rats raised by Mardones (1960). The P and NP lines have now been propagated to the 13th generation. The merits and limitations of such selectively bred animals compared with those of inbred strains developed by mating among siblings without regard to phenotype (except those related to reproductive fitness) have been discussed previously (Eriksson 1971).

Preference testing of the P and NP lines has also been performed as a function of the concentration of ethanol. By this method of testing, ethanol preference is defined by the intake of more than 50 percent of the total fluid volume as the ethanol solution. Myers and Veale (1972) showed that rats will usually prefer solutions of ethanol over water when ethanol concentration is lower than 6 percent. However, rats of the P line, both in the S_8 and S_{12} generations, exhibited high preference for ethanol solutions even when concentration was 14 percent or greater (figure 3). By contrast, the NP animals

Figure 1. **Daily Ethanol Consumption of P_1 and S_1 Rats in Free-Choice Testing of Preference Between a 10 Percent Solution (v/v) of Ethanol and Water**



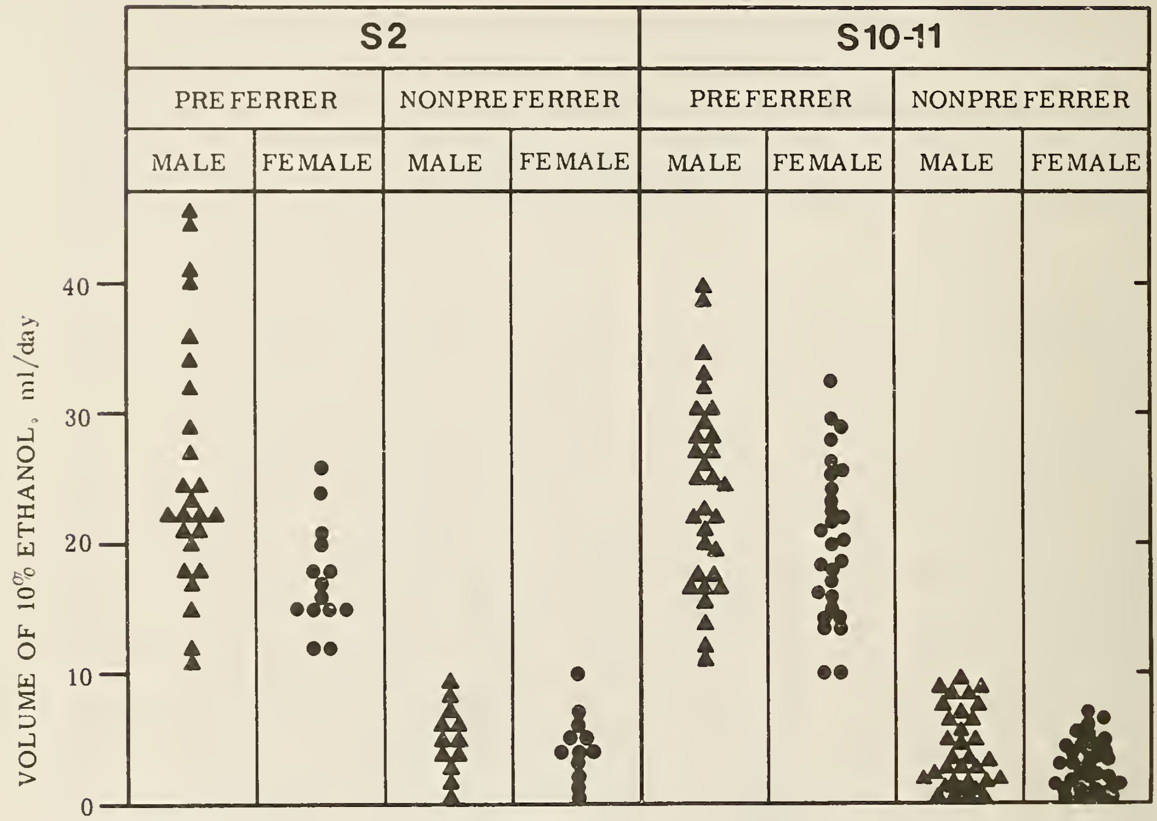
P_1 is the foundation stock, and S_1 is the first generation of the P and NP lines. The mean and SEM of the volumes in milliliters of 10 percent ethanol consumed daily are listed for each of the subgroups.

Adapted with the publisher's permission from Li et al. 1979. Copyright 1979 by Elsevier Sequoia S.A.

never exhibited preference for ethanol even when concentration was as low as 2 percent. Therefore, the phenotypes of the lines are readily definable either by the standard two-bottle preference test that employs a single (10 percent) ethanol concentration or by the method of testing for preference over a range of ethanol concentrations.

The animals under free-choice testing between 10 percent ethanol and water, with food available ad libitum, substitute ethanol calories for food calories. Their body weights are maintained at the expected levels (Lumeng et al. 1977). A comparison of the daily voluntary

Figure 2. **Daily Ethanol Consumption of S_2 and $S_{10\text{-}11}$ Rats in Free choice Testing of Preference Between a 10 Percent Solution (v/v) of Ethanol and Water**



MEAN	24.8	17.4	5.0	4.3	23.9	20.2	3.7	2.8
SEM	2.0	1.1	0.7	0.7	1.3	1.1	0.5	0.3
n	25	14	12	12	31	28	33	34

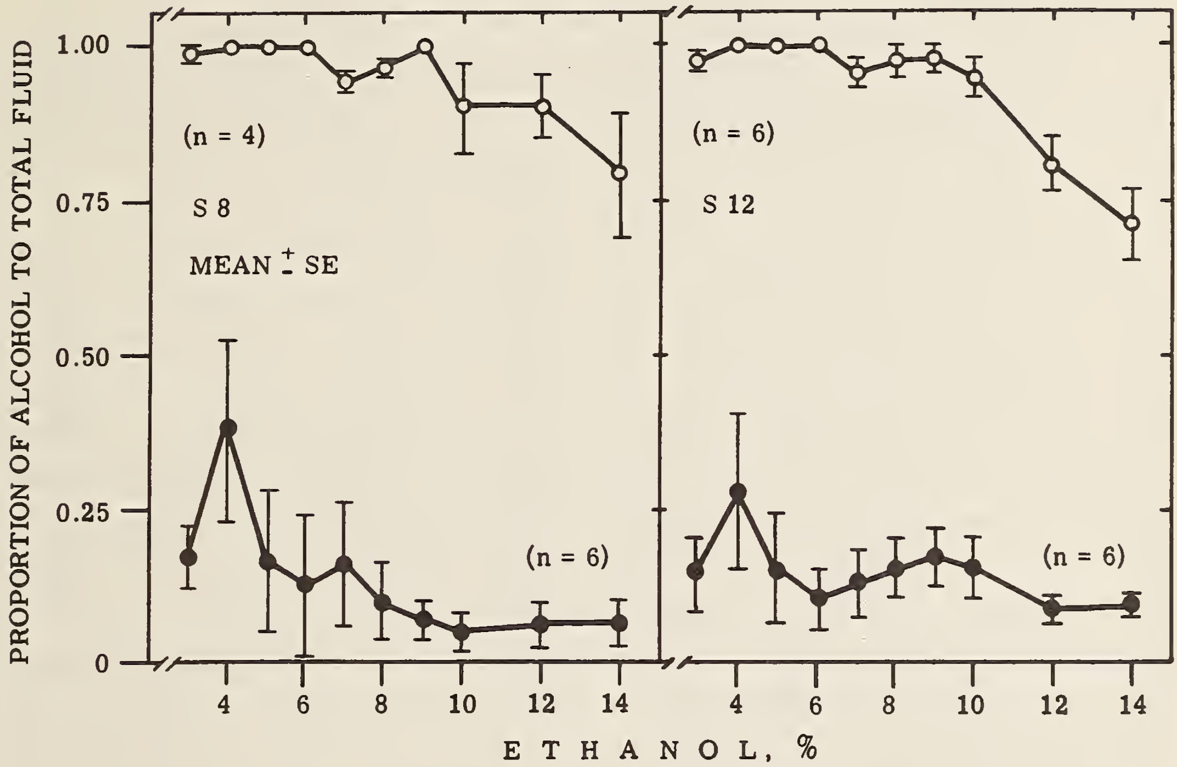
S_2 is the second filial generation of the selectively bred P and NP lines, and $S_{10\text{-}11}$ designates the 10th and 11th generations. The mean and SEM of the volumes in milliliters of 10 percent ethanol consumed daily are listed for each of the subgroups.

Adapted with the publisher's permission from Li et al. 1979. Copyright 1979 by Elsevier Sequoia S.A.

ethanol consumption of the P line of rats (6.3 ± 1.7 g/kg/day) with ethanol-metabolizing capacity has been made: The mean ethanol elimination rate of fed animals given 1.5-2 g ethanol/kg body weight by intraperitoneal injection is 7-9 g/kg body weight/day, and that for 12-hour fasted animals is 5-7 g/kg/day. Therefore, the amount of ethanol consumed by a very substantial number of the animals in the P line is close to their measured metabolic limit.

To ascertain whether heterogeneity in voluntary drinking behavior exists in other stocks of Wistar rats, preference testing has been performed with a colony from Purdue University and another from a commercial supply house (Laboratory Supply Company, Indianapolis,

Figure 3. **Comparison of the Drinking Behaviors of the P and NP Lines in the S_8 and S_{12} Generations**

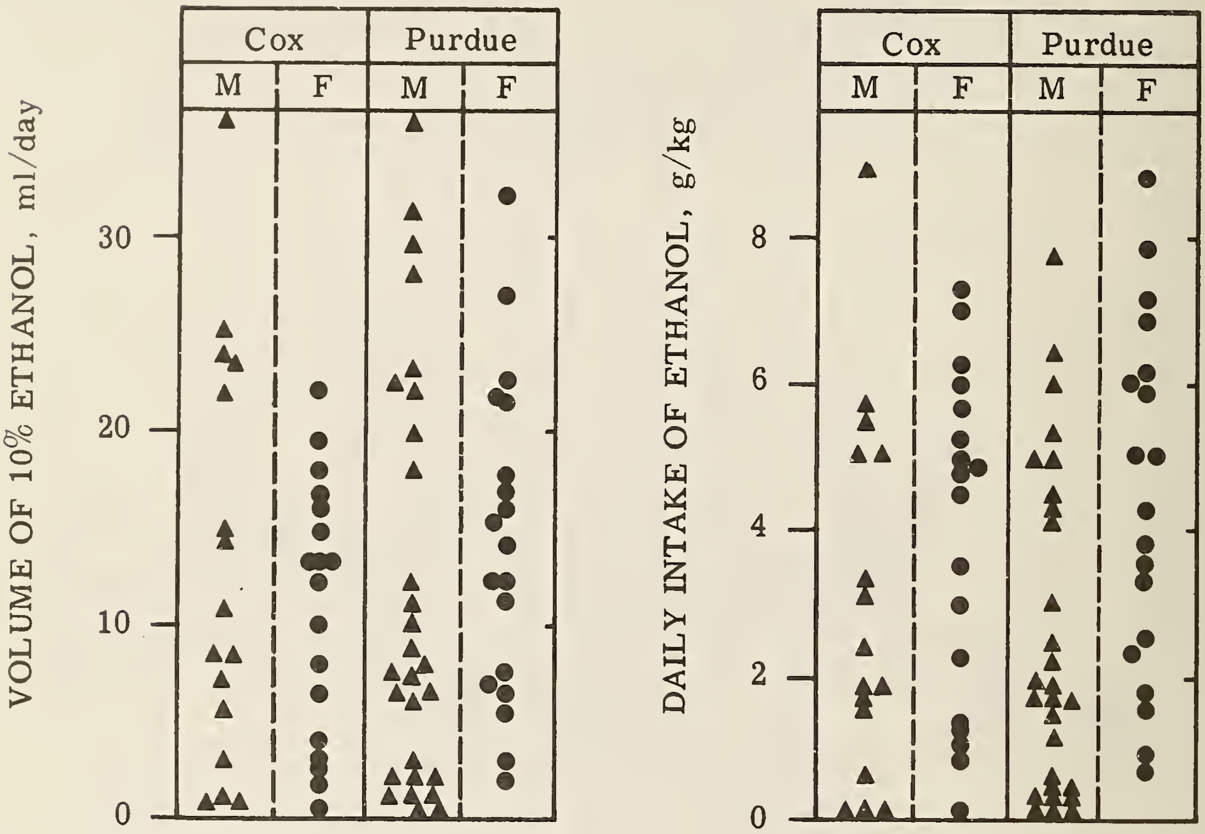


The preference between water and a solution of ethanol is plotted as a function of ethanol concentration. P lines, 0 0; NP line, ● ●

Adapted with the publisher's permission from Li et al. 1979. Copyright 1979 by Elsevier Sequoia, S.A.

Ind.). As before, the free-choice consumption of 10 percent ethanol and water was measured while food was available ad libitum (figure 4). The degree of heterogeneity in both these colonies is similar to that of the Walter Reed colony (figure 1) and many animals were found to consume 7 g or more ethanol/kg body weight per day. Therefore, animals exhibiting voluntary ethanol consumption in amounts approaching their metabolic limit can be found in common stock Wistar rats from different sources. Such animals should be suitable for similar experiments on selective breeding for alcohol preference.

Figure 4. **Daily Ethanol Consumption of Other Stocks of Wistar Rats in Free-Choice Testing of Preference Between a 10 Percent Solution (v/v) of Ethanol and Water**



Cox refers to Wistar rats from a commercial animal supply house; Purdue refers to Wistar rats from Purdue University.

Ethanol and Acetaldehyde Metabolism in the Alcohol-Preferring and Alcohol-Nonpreferring Lines of Rats

Ethanol and acetaldehyde metabolism in the P and NP lines has been studied with animals in the S_8 generation (Li and Lumeng 1977). Although there was a significant difference between the sexes in the rate of ethanol elimination in vivo, no difference between the lines was observed in either sex. Furthermore, it was found that blood acetaldehyde levels following the administration of ethanol (1.6 g/kg body weight, i.p.) did not differ between the lines, although a difference between the sexes was again observed. Because the ethanol consumption scores of male and female rats in each of the lines were similar, it was concluded that ethanol elimination rates and blood acetaldehyde levels are not related to ethanol preference or nonpreference in these lines. Studies in vitro confirmed the absence of a correlation between ethanol preference and hepatic ethanol metabolism (Li and Lumeng 1977).

The blood ethanol and acetaldehyde concentrations attained in P rats consuming 6-7 g ethanol/kg body weight per day during free choice have also been compared with those in NP rats drinking less than 1.5 g/kg/day under the same conditions (Li and Lumeng 1977). Four measurements during the dark cycle, when more than 70 percent of the fluid intake occurs, showed blood ethanol concentrations ranging from 5 to 74 mg percent in the P rats but 0.5 mg percent or less in the NP rats. Acetaldehyde was detected in the blood of the P animals in at least two-thirds of the samples, whereas it was below the limit of detection in all blood samples from the NP rats. These studies, therefore, demonstrate that the natural pattern of free-choice consumption of ethanol by the preferring line is sufficient to elevate blood ethanol concentrations to substantial levels at least during the dark cycle. They further suggest that acetaldehyde is not directly responsible for the alcohol aversion of the NP rats, because it was below the limit of detection in their blood during free-choice consumption. It has not been ruled out, however, that acetaldehyde may have caused a conditioned taste aversion in the NP animals as a result of the initial period of forced ethanol consumption during preference testing.

Effect of Ethanol Consumption on the Content of Brain Neurotransmitter and Related Compounds in Alcohol-Preferring and Alcohol-Nonpreferring Rats

Because the P rats did not show signs of intoxication during free-choice consumption of ethanol, the question arose whether ethanol, in the amounts consumed and over a period of time, exerts a pharmacologic effect. Hence, the effect of oral ethanol consumption on brain neurotransmitter content was examined (Penn et al. 1978). In this study, male P and NP rats were first tested for alcohol preference for 3 weeks by the usual procedure. Those consuming more than 5 g/kg/day in the P line and less than 1.5 g/kg/day in the NP line were selected for study. These animals were then divided into two subgroups: One received 10 percent ethanol and the other received water as the sole source of fluid for 1 week. During this 1-week period of forced ethanol consumption, the average ethanol intake of the P animals was 7.4 g/kg/day, approximately equal to their oral intake under free-choice conditions. The average intake for the NP animals was 6.6 g/kg/day. After this period, the animals were killed by the near-freeze method of Takahashi and Aprison (1964), the brains were dissected, and the contents of 11 neurotransmitter and related compounds in the cerebellum, telencephalon, and diencephalon-mesencephalon were analyzed (Smith et al. 1975). These compounds were serotonin, 5-hydroxyindoleacetic acid, tyrosine, norepinephrine, acetylcholine, GABA, glutamate, aspartate, glycine, alanine, and taurine.

No difference was found in the cerebellum between the ethanol-treated and water-control subgroups in either the P or the NP line. However, differences in the content of several compounds were observed in the diencephalon-mesencephalon and in the telencephalon. In the P animals, the level of tyrosine was elevated in the diencephalon-mesencephalon and GABA level was higher in the telencephalon of the ethanol-treated subgroup compared with the water subgroup. In the NP animals, tyrosine was also elevated in the diencephalon but serotonin was elevated in the telencephalon of the ethanol-treated subgroup relative to the control subgroup (Penn et al. 1978). These data indicate that ethanol in an amount comparable to that consumed by the P line of rats under conditions of free choice is capable of altering neurotransmitter metabolism in the brain. By this criterion, therefore, the free-choice consumption of ethanol should be exerting a CNS pharmacologic effect in the P line of rats.

Innate Difference in CNS Sensitivity to the Effects of Ethanol Between Alcohol-Preferring and Alcohol-Nonpreferring Rats

A number of recent studies have shown that alcohol-preferring animals are less sensitive to the hypnotic and sedative effects of ethanol than are alcohol-nonpreferring animals. This has been shown by studies of sleep time in the inbred C57BL and DBA mouse strains (Kakihana et al. 1966; Randall and Lester 1974) and by sleep time and tilting plane studies in the selectively bred AA and ANA rats (Nikander and Pekkanen 1977; Rusi et al. 1977). This relative insensitivity of the alcohol-preferring animals to the acute effects of ethanol has been called innate tolerance by some investigators. We have recently found that the P and NP lines exhibit a similar difference in sensitivity to ethanol. When male P and NP rats were given a hypnotic dose of ethanol, 2.5 g/kg body weight intraperitoneally, a greater hypothermic effect was produced in the NP animals than in the P animals (figure 5). In accord with this finding, the sleep time with the same dose of ethanol was longer and blood ethanol concentration at the time of awakening was significantly lower in NP than in the P animals (table 3). As already mentioned, P and NP rats of the same sex did not differ significantly in their rates of ethanol elimination. Thus a correlate common to all three alcohol preference animal models is innate CNS tolerance or insensitivity to the sedative and hypnotic effects of ethanol. Preliminary studies have also shown that the P and NP lines differ innately in their brain content of neurotransmitter and related compounds (Penn et al. 1978) and in grooming activity and ambulation scores upon open-field testing (Li et al. 1979).

Table 3. Sleep Time and Blood Alcohol Concentration on Awakening: P vs. NP Rats (Mean ± S.D.)

Strain	*N*	Sleep Time [1, 2] (min)	Blood Ethanol (mg %)
NP	5	155 ± 76	162 ± 24
P	5	81 ± 59	214 ± 7

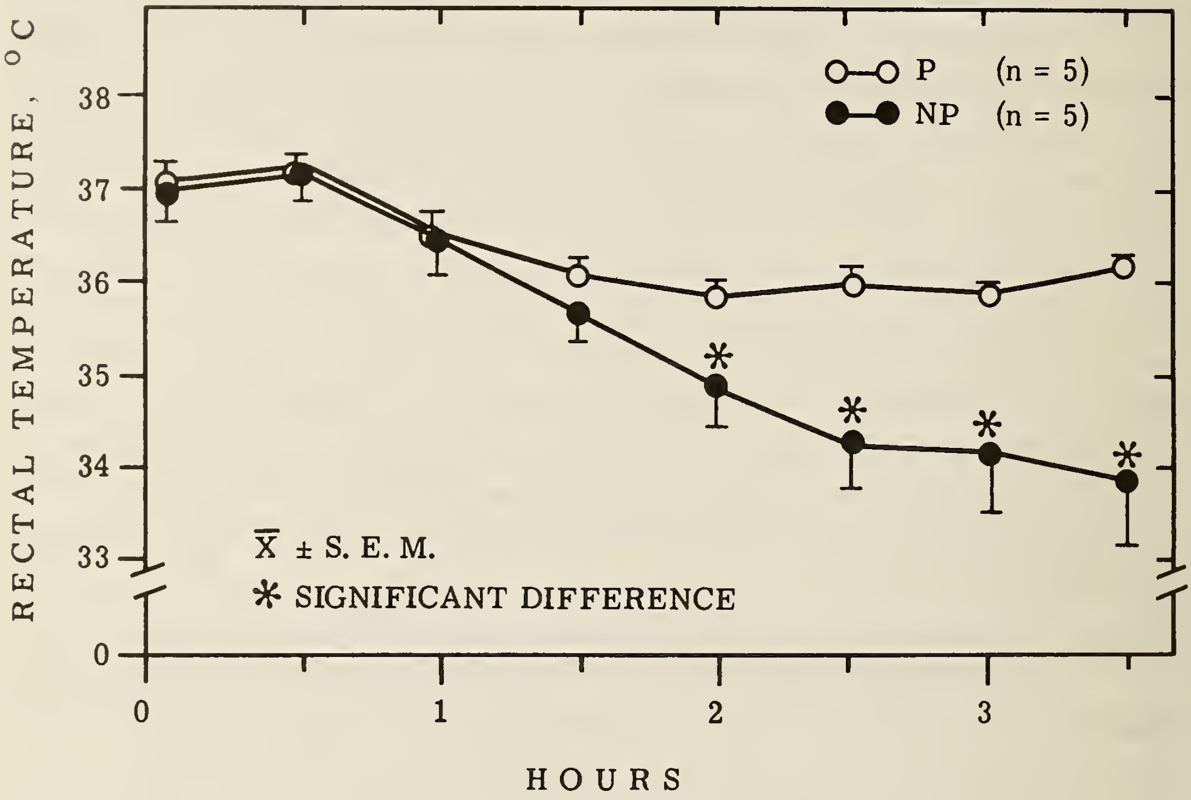
[1] P vs. NP was found to be significant at $p < 0.005$.

[2] Sleep time was measured following the intraperitoneal injection of 2.5 g ethanol/kg body weight.

The Reinforcing Properties of Ethanol for Alcohol-Preferring Rats

We have recently reported that free-feeding rats of the P line, trained to bar press for reward, will work in an operant situation to obtain 10 percent ethanol orally in the presence of unlimited amounts of food and water. Indeed, the response to the reinforcement ratio can be raised to 6 or 7 before water intake increases, yielding a response rate of over 1,000 bar presses/24 hours in each of the animals tested (Mardones 1960). Sinclair (1974) also demonstrated by operant techniques that ethanol is a positively reinforcing substance for alcohol-preferring rats from the AA, ANA, and Sprague-Dawley

Figure 5. Comparison of the Hypothermic Effects of Ethanol Injection upon Male Rats of the P and NP Lines



A hypnotic dose of ethanol, 2.5 g/kg body weight, was injected intraperitoneally at various times prior to measurement of rectal temperature.

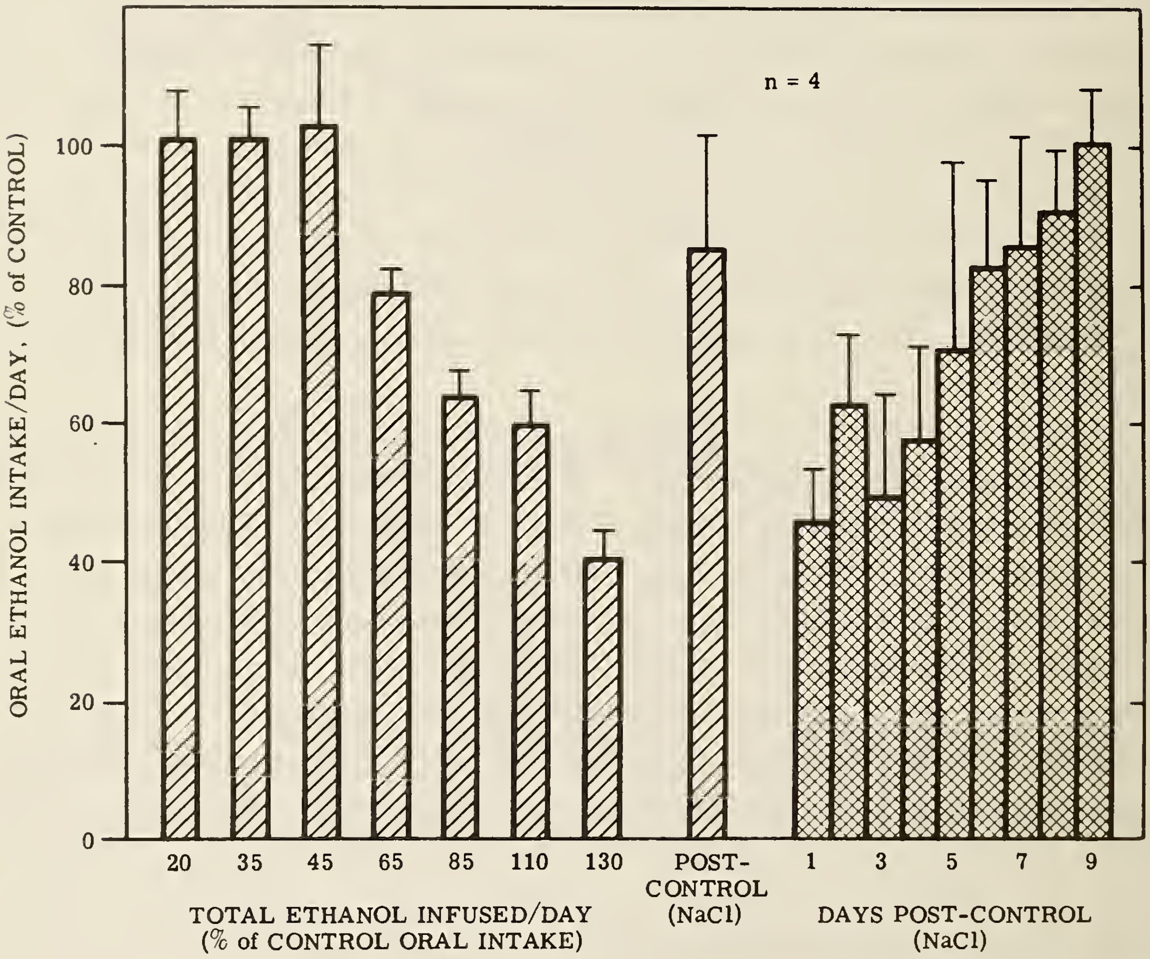
Reproduced with the publisher's permission from Li et al. 1979. Copyright 1979 by Elsevier Sequoia S.A.

strains. Because food and water were freely available to the test animals in both these studies, it appears unlikely that alcohol-preferring rats found the ethanol solutions rewarding because of caloric needs or thirst.

The above studies, however, do not distinguish whether the reinforcing properties of ethanol are due to its pharmacologic or orosensory effect. In an attempt to dissociate these factors, the effect of intravenously administered ethanol on free-choice oral consumption of 10 percent ethanol was examined in male P rats (Waller et al. 1978). Following a preinfusion control period, a polyethylene cannula was implanted in the external jugular vein according to the procedure of Pickens and Dougherty (1972). Hourly infusions of ethanol (0.1 milliliters over 10 seconds) were given on each day totaling 20 to 130 percent of the daily voluntary oral ethanol intake of each of the animals. Throughout the experiment, 10 percent ethanol, water, and food were available ad libitum. When 65 percent or more of the mean daily intake of ethanol was administered intravenously, oral ethanol consumption decreased dramatically (figure 6). Concomitant with these changes, oral water intake increased 5-30 times. Recovery of the usual drinking behavior occurred within 9 days postinfusion. During the ethanol infusion period, the intake of solid food decreased only slightly, but it is known that these rats will substitute ethanol calories for food calories (Lumeng et al. 1977) and body weights remained stable. These results are consistent with the hypothesis that the free-choice drinking of ethanol in P rats is controlled, at least in part, by the metabolic capacity of the animal for ethanol and by blood ethanol concentrations. They also suggest that, although orosensory cues may be present, the pharmacologic effect of ethanol is an important determinant of ethanol drinking behavior in these rats.

In order to dissociate completely the orosensory from the metabolic and pharmacologic properties of ethanol as factors in ethanol preference, we recently began studies to determine whether rats of the P line would self-administer ethanol intravenously using operant techniques. The study design and the results in six animals are shown in table 4. The rats were trained to bar press for 10 percent ethanol while water and food were available ad libitum. Using a fixed-ratio schedule, the response-reinforcement ratio (FR) was raised to 10 and then decreased to 5. The number of reinforcements per day at FR5 for the drinking of 10 percent ethanol as reward was about 6 times that for water as reward. An intravenous catheter was then implanted into the external jugular vein and the rate of responding for drinking water when coupled with an infusion of ethanol was compared with the rate for drinking water coupled with an infusion of saline. Infusions were given in 0.1 milliliter pulses over 4 seconds. At each of the doses of ethanol studied, the animal bar pressed more frequently for drinking water in the dipper when ethanol was infused than when saline was infused. These studies, therefore, strongly suggest that the pharmacologic effect of ethanol contributes to the alcohol-seeking behavior of these rats.

Figure 6. Effect of Intravenous Ethanol Infusion upon Voluntary Oral Ethanol Intake of Rats from the P Line



Ethanol was infused at hourly intervals (24 doses/day). Daily consumption of 10 percent ethanol in the presence of water and food ad libitum was measured.

Reproduced with the publisher's permission from Li et al. 1979. Copyright 1979 by Elsevier Sequoia S.A.

Table 4. Intravenous Self-Administration of Ethanol by Rats from the P Line When Food Is Freely Available

Solution in the Dipper	Solution Available Ad Libitum	Ethanol/0.1 ml Infusion (mg/kg body wt)	Reinforcements per Day at FR5
10% ethanol	water	none	212 ± 15[1]
water	10% ethanol	none	35 ± 6
10% ethanol	water	saline only	171 ± 13
water	10% ethanol	saline only	260 ± 5
water	water	6	106 ± 7
water	water	9	89 ± 5
water	water	12	64 ± 4

[1] $N = 6$, Mean ± SEM.

Production of Physical Dependence in Weight-Reduced Alcohol-Preferring Rats

We showed previously that caloric restriction in combination with the flavoring of ethanol solutions with sucrose can enhance the voluntary ethanol consumption in the P line to about 10 g/kg/day (Lumeng et al. 1978). Under these conditions, blood ethanol concentrations become continuously elevated. This occurs in part because of the weight reduction but also because both fasting and weight reduction decrease ethanol elimination rates and liver alcohol dehydrogenase activity (Lumeng et al. 1978). We have examined whether this kind of regimen on a chronic basis will produce dependence and withdrawal. Reduction of body weight to 80 percent of the animals' free-feeding weight increased ethanol intake during free choice from about 7 to 10 g/kg/day. The addition of saccharin (0.125 percent) and NaC1 (1 percent) further increased intake to about 12 g/kg/day. After a total of 40 days, 20 at each of these two levels of intake, ethanol was withdrawn and the animals tested for signs of the abstinence syndrome.

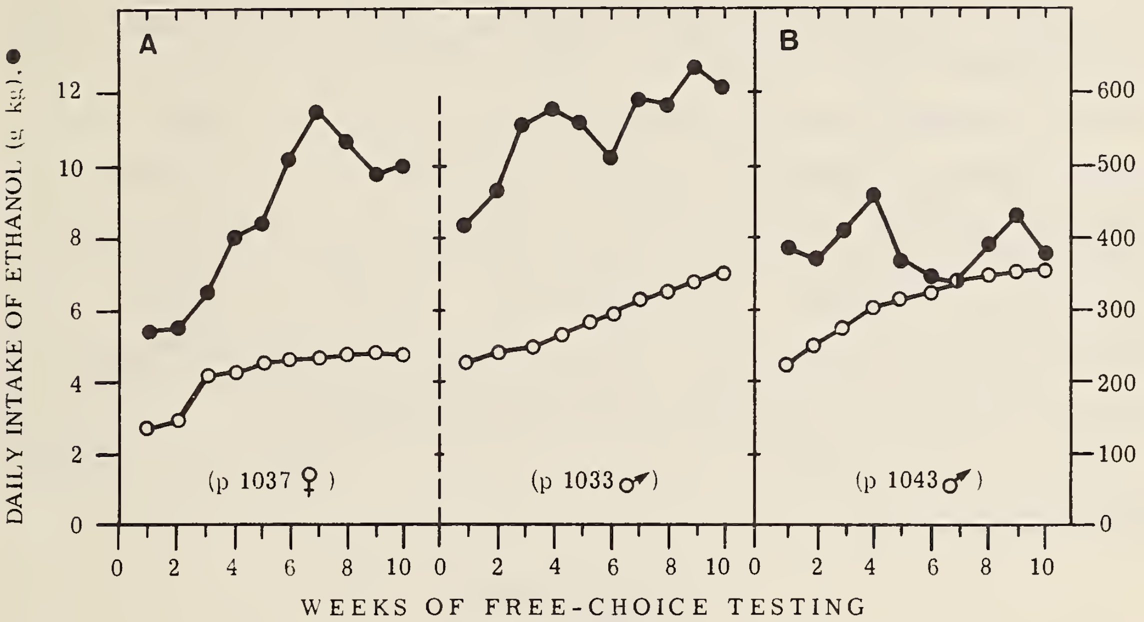
Withdrawal signs were scored by the behavioral rating scale of Hunter et al. (1975), by open-field activity testing as described by Cicero et al. (1971), and by activity in a runway apparatus as described by Pohorecky (1976). Behavioral signs of withdrawal (stages III to V) were observed in five of six animals in the first 24 hours with two animals exhibiting seizures induced by 100 decibels of noise from an electric bell. Subsequently, impaired activity in the runway appeared in four of six animals; in open-field testing, one animal exhibited hypoactivity while four animals exhibited hyperactivity. By these measurements, therefore, at least two-thirds of the animals had developed physical dependence on ethanol as the result of consuming 10-12 g ethanol/kg body weight/day for 40 days.

The Appearance of Tolerance in Free-Feeding Alcohol-Preferring Rats Following Prolonged Voluntary Ethanol Consumption

We had previously determined in alcohol-naive, postpubertal Wistar rats that the ethanol elimination rate of fasted and weight-reduced animals is about 5 or 6 g/kg/day (Lumeng et al. 1978). The amount of ethanol consumed by the weight-reduced P rats that ultimately exhibited signs of physical dependence, 10-12 g/kg/day, greatly exceeded the ethanol elimination rates of alcohol-naive rats, suggesting that they had also developed tolerance. To ascertain whether P rats under free-feeding conditions would also exhibit enhanced drinking with prolonged exposure, 12 animals were put on free-choice testing with 10 percent ethanol and water for 10 weeks. Two patterns of intake emerged. In 4 of the 12 animals, ethanol intake remained constant over time (figure 7B). On the other hand, 8 of the animals exhibited a progressive rise in ethanol consumption, reaching 10-12 g/kg/day after about 6 weeks (figure 7A). Our previous studies had indicated that the ethanol elimination rate of free-feeding, postpubertal P and NP rats not chronically exposed to ethanol is about 7-9 g/kg/day (Li and Lumeng 1977). Because the amount of ethanol consumed by two-thirds of the rats with prolonged free-choice testing consistently exceeded this amount by a large margin, it appears that metabolic tolerance had developed in these but not all the P animals.

As a beginning to the assessment of the relative roles of metabolic versus neuronal tolerance in the enhanced free-choice drinking of these rats, we measured the blood alcohol levels at two points in the dark cycle wherein most of the food and ethanol is consumed. These data and those from the weight-reduced rats, also consuming about 10-12 g/kg/day, are compared in table 5. The mean and range of

Figure 7. Effect of Prolonged Free-Choice Testing upon Ethanol Intake of Rats from the P Line



Reproduced with the publisher's permission from Li et al. 1979. Copyright 1979 by Elsevier Sequoia S.A.

blood ethanol concentrations in the two groups were similar and most values were below 100 mg percent. The data suggest that metabolic tolerance represents a major component of the adaptive response to chronic ethanol ingestion in these animals. Moreover, because similar amounts of ethanol ingested and in the blood of the weight-reduced animals produced physical dependence, it is possible that dependence can occur in the free-feeding P line of rats with the prolonged voluntary consumption of 10 percent ethanol. Studies to test this hypothesis are in progress.

Table 5. Blood Alcohol Concentrations in Rats from the P Line During Free-Choice Drinking of 10 Percent Ethanol and Water: Ad Libitum vs. 80 Percent Weight-Reduced Feeding Regimens

	Blood Ethanol Concentration (mg%)	
	Mean	Range
Fed Ad Libitum ($N=6$)		
2.5 hours into dark cycle[1]	41	2-79
10.5 hours into dark cycle	58	19-105
Weight-reduced ($N=5$)		
2.5 hours into dark cycle	68	2-97
10.5 hours into dark cycle	75	21-105

[1] The light-dark cycles were comprised of 12 hours each per day.

Reproduced with the publisher's permission from Li et al. 1979. Copyright 1979 by Elsevier Sequoia, S.A.

Summary and Conclusions

Our studies to date on a line of rats selectively bred for alcohol preference have revealed the following important features that indicate its potential usefulness as an animal model of alcoholism (presumably, other animals similarly bred would also be suitable). (1) The drinking is voluntary and not contingent on caloric restriction or thirst. (2) The amount of ethanol voluntarily consumed by them approaches their apparent maximum capacity for ethanol elimination. (3) This amount of ethanol is capable of altering brain neurotransmitter content and hence exerts a CNS pharmacologic effect. (4) The animals will work to obtain ethanol even when food and water are freely available; in this sense, they exhibit psychological or behavioral dependence. (5) The animals are innately insensitive or tolerant to the hypnotic or sedative effects of ethanol. (6) The animals will work by bar pressing also for the intravenous administration of ethanol. (7) With prolonged free-choice consumption, ethanol intake increases to as much as 12 g/kg body weight/day without producing other behavioral deficits, suggesting the development of tolerance.

Inasmuch as signs of intoxication do not appear spontaneously, it might be interpreted that this kind of drinking is still "controlled" in these animals. The important questions at this juncture, therefore, are, What factors regulate this drinking behavior and what conditions can cause them to be overridden? In this regard, we have already observed that, with operant responding, episodes of acute intoxication occur as evidenced by loss of consciousness and that, when the animals are calorically restricted, physical dependence develops. Thus it should be possible to discern what environmental factors singly or in combination can produce aberrancy in the drinking behavior of these animals. Furthermore, the role of various biologic processes, e.g., tolerance and dependence, in the development and maintenance of aberrant drinking behavior can be investigated. Such a model, therefore, should provide a means to study the interaction of genetic, metabolic, and environmental factors in the production of alcoholism, experiments that are not possible in the human.

References

Ahtee L. The metabolism of brain 5-hydroxytryptamine in rat strains selected for alcohol preference or rejection; effect of ethanol. In: Forsander, O., and Eriksson, K., eds. *Biological Aspects of Alcohol Consumption.* Vol. 20. Helsinki: Finnish Foundation for Alcohol Studies, 1972. pp. 193-199.

Blass, J.B., and Gibson, G.E. Abnormality of a thiamine-requiring enzyme in patients with Wernicke-Korsakoff syndrome. *New Engl J Med,* 297:1367-1370, 1977.

Cadoret, R.J., and Gath, A. Inheritance of alcoholism in adoptees. *Br J Psychiatry,* 132:252-258, 1978.

Cicero, T.J., and Smithloff, B.R. Alcohol oral and self-administration in rats: Attempts to elicit excessive intake and dependence. *Adv Exp Med Biol,* 35:213-224, 1973.

Cicero, T.J.; Snider, S.R.; Perez, V.J.; and Swanson, L.W. Physical dependence on and tolerance to alcohol in the rat. *Physiol Behav,* 6:191-198, 1971.

Eriksson, K. Rat strains specially selected for their voluntary alcohol consumption. *Ann Med Exp Biol Fenn,* 49(2):67-72, 1971.

Eriksson, K. Behavioral and physiological differences among rat strains specially selected for their alcohol consumption. *Ann NY Acad Sci,* 197:32-41, 1972.

Eriksson, K., and Närhi, M. Specially selected rat strains as a model of alcoholism. In: Spiegel, A., ed. *The Laboratory Animal in Drug Testing.* Stuttgart: Gustav Fischer Verlag, 1972. pp. 163-171.

Goodwin, D.W.; Schulsinger, F.; Hermansen, L.; Guze, S.B.; and Winokur, G. Alcohol problems in adoptees raised apart from alcoholic biological parents. *Arch Gen Psychiatry,* 28:238-243, 1973.

Hunter, B.E.; Riley, J.N.; Walker, D.W.; and Freund, G. Ethanol dependence in the rat: A parametric analysis. *Pharmacol Biochem Behav,* 3:619-629, 1975.

Kakihana, R.; Brown, D.R.; McClearn, G.E.; and Tabershaw, I.R. Brain sensitivity to alcohol in inbred mouse strains. *Science,* 154:1574-1575, 1966.

Lester, D., and Freed, E.X. Theoretical review criteria for an animal model of alcoholism. *Pharmacol Biochem Behav*, 1:103-107, 1973.

Li, T.-K., and Lumeng, L. Alcohol metabolism of inbred strains of rats with alcohol preference and nonpreference. In: Thurman, R.G.; Williamson, J.R.; Drott, H.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems*. Vol. 3. New York: Academic Press, 1977. pp. 625-633.

Li, T.-K.; Lumeng, L.; McBride, W.J.; Waller, M.B.; and Hawkins, T.D. Progress toward a voluntary consumption model of alcoholism. *Drug Alcohol Depend*, 4:45-60, 1979.

Lumeng, L.; Bosron, W.F.; and Li, T.-K. Quantitative correlation of ethanol elimination rates in vivo with liver alcohol dehydrogenase activities in fed, fasted and food-restricted rats. *Biochem Pharmacol*, 28:1547-1551, 1979.

Lumeng, L.; Hawkins, T.D.; and Li, T.-K. New strains of rats with alcohol preference and nonpreference. In: Thurman, R.G.; Williamson, J.R.; Drott, H.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems*. Vol. 3. New York: Academic Press, 1977. pp. 537-544.

Lumeng, L.; Penn, P.E.; Gaff, T.M.; Hawkins, T.D.; and Li, T.-K. Further characterization of a new rat strain with high alcohol preference. In: Seixas, F.A., ed. *Currents in Alcoholism*. Vol. 3. New York: Grune & Stratton, 1978. pp. 23-35.

Mardones, J. Experimentally induced changes in the free selection of ethanol. *Int Rev Neurobiol*, 2:41-76, 1960.

Myers, R.D., and Veale, W.L. The determinants of alcohol preference in animals. In: Kissin, B., and Begleiter, H., eds. *The Biology of Alcoholism*. Vol. 2. New York: Plenum, 1972. pp. 131-168.

National Council on Alcoholism, Criteria Committee. Criteria for the diagnosis of alcoholism. *Ann Intern Med*, 77:249-258, 1972.

Nikander, P., and Pekkanen, L. An inborn alcohol tolerance in alcohol-preferring rats. The lack of relationship between tolerance to ethanol and the brain microsomal (Na_+K_+) ATPase activity. *Psychopharmacology*, 51:219-223, 1977.

Partanen, J.; Bruun, K.; and Markkanen, T. *Inheritance of Drinking Behavior: A Study on Intelligence, Personality, and Use of Alcohol in Adult Twins*. Vol. 14. Helsinki: Finnish Foundation for Alcohol Studies, 1966.

Penn, P.E.; McBride, W.J.; Lumeng, L.; Gaff, T.M.; and Li, T.-K. Neurochemical and operant behavioral studies of a strain of alcohol-preferring rats. *Pharmacol Biochem Behav*, 8:475-481, 1978.

Pickens, R., and Dougherty, J. A method for chronic intravenous infusion of fluids in unrestrained rats. Reports from Research Laboratories. PR-72-1. Minneapolis: Department of Psychiatry, University of Minnesota, 1972.

Pohorecky, L.A. Withdrawal from ethanol: Simple quantitative behavioral tests for its evaluation. *Psychopharmacology*, 50:125, 1976.

Randall, C.L., and Lester, D.J. Differential effects of ethanol and phenobarbitol on sleep time in C57BL and BALB mice. *J Pharmacol Exp Ther*, 188:27-33, 1974.

Rusi, M.; Eriksson, K.; and Mäki, J. Genetic differences in the susceptibility to acute ethanol intoxication in selected rat strains. In: Gross, M.M., ed. *Alcohol Intoxication and Withdrawal*. Vol. IIIA. New York: Plenum, 1977, pp. 97-109.

Sinclair, J.D. Rats learning to work for alcohol. *Nature*, 249:590-591, 1974.

Smith, J.E.; Lane, J.D.; Shea, P.A.; McBride, W.J.; and Aprison, M.H. A method for concurrent measurement of picomole quantities of acetylcholine, choline, dopamine, norepinephrine, serotonin, 5-hydroxytryptophan, 5-hydroxyindoleacetic acid, tryptophan, tyrosine, glycine, aspartate, glutamate, alanine, and gamma-aminobutyric acid in single tissue samples from different areas of rat central nervous system. *Anal Biochem*, 64:149-169, 1975.

Takahashi, R., and Aprison, M.H. Acetylcholine content of discrete areas of the brain obtained by near-freezing method. *J Neurochem*, 11:887-898, 1964.

Vesell, E.S.; Page, J.G.; and Passananti, G.T. Genetic and environmental factors affecting ethanol metabolism in man. *Clin Pharmacol Ther*, 12:192-201, 1971.

Waller, M.B.; McBride, W.J.; Lumeng, L.; and Li, T.-K. Production of physical dependence in the alcohol-preferring and nonpreferring lines of rats. *Neurosci Abstr*, 4:136, 1978.

Wolff, P.H. Ethnic differences in alcohol sensitivity. *Science*, 175:449-450, 1972.

Selective Breeding for Ethanol Sensitivity: Least Affected and Most Affected Rats[*]

M.B. Bass and D. Lester[**]

The widespread consumption of alcoholic beverages is undoubtedly related to the intoxicating properties of ethanol. Because pharmacological responses to ethanol are likely to influence patterns of alcohol consumption, it seems reasonable to postulate that these reactions contribute to the etiology of alcoholism. For this reason we have chosen to breed rats selectively on the basis of sensitivity to the pharmacological effects of ethanol.

Practical considerations demand that the response chosen as the phenotype for selective breeding be quantifiable and sensitive to ethanol and not require any substantial amount of pretraining. The response chosen was ethanol-induced changes in locomotor activity. Conceptually, this breeding project is similar to that of McClearn and Kakihana (1973), which led to the development of long-sleep (LS) and short-sleep (SS) mice, selectively bred for the duration of ethanol-induced narcosis. Rats rather than mice, however, are used in our studies, and the phenotype is a response to ethanol observed in conscious animals at lower blood alcohol levels, more similar to those attained during moderate intoxication in humans.

---

[*] Supported in part by grants AA-00216 and AA-01849 from the National Institute on Alcohol Abuse and Alcoholism to D. Lester, by a grant from the Veterans Administration to E.X. Freed, and by grants from the Biomedical Sciences Research Grant and the Busch Memorial Fund of Rutgers University to D. Lester.

[*] This project has greatly benefited in all phases from the dedicated and competent assistance of Elizabeth Haugen.

Method

Subjects

The original breeding stock consisted of 16 Sprague-Dawley albino and 16 Long-Evans hooded rats obtained from Blue Spruce Farms, Altamont, N.Y. Both strains have substantial genetic heterogeneity, and the use of two strains as a foundation stock would be expected to increase the range of response, which was large on initial screening (Riley et al. 1976). Animals were generally housed three to five per case under a normal 12-hour light-dark cycle. Food was available ad libitum except for 24 hours before testing; water was always available.

Apparatus

Locomotor activity was measured on Lafayette A501 activity platforms. Platform activity moved a magnet mounted vertically on the underside of each platform, inducing a current in a surrounding coil; movements generating signals above threshold amplitude were recorded. Beginning with the ninth generation, electronic integrators were added to utilize all signals from the coil. The platform, 30.5 centimeters square, was enclosed from the sides and above.

Procedure

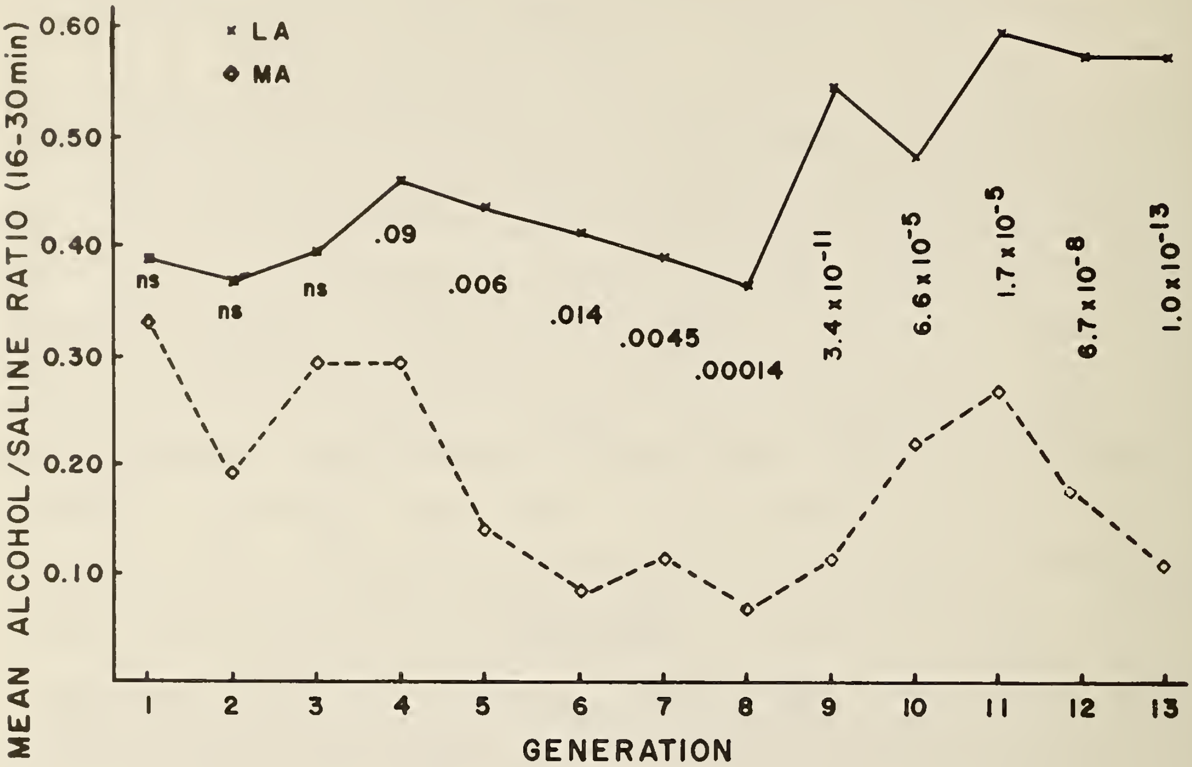
Activity testing was conducted in the dark. After a 24-hour fast, the rats were exposed to the activity chamber for 75 minutes to allow habituation to the test environment. The experiment proper began 7 days later. Rats of both sexes and strains, 24-hours fasted, received an intraperitoneal injection (15 ml/kg) of ethanol in one of four doses: 0.0 (saline), 0.75, 1.50, and 2.25 g ethanol/kg. Rats were placed in an activity chamber for 75 minutes; activity counts were cumulated at 15-minute intervals. Each rat was tested after each of the four doses at weekly intervals in a random order following the habituation session. The experiment was conducted in two replications of 16 rats, each consisting of four members of each sex and strain. Rats from each replication were bred independently until the replications were combined in the F_3generation.

The dose-response experiments indicated that ethanol at 0.75 g/kg did not reliably alter locomotor activity, but 1.50 and 2.25 g/kg depressed activity from 16 to 60 minutes after injection (Riley et al. 1976). Because the greatest ethanol-induced decrement in activity was between 0.75 and 1.5 g/kg, and because 2.25 g/kg produced only slightly greater depression than 1.5 g/kg, the latter was chosen as the dose for testing and selective breeding. Rats from each replication were retested beginning 15 minutes after 15 ml/kg isotonic saline and again 1 week later after 1.5 g ethanol/kg i.p., given as a 10 percent w/v solution in saline. A "most" affected rat and a "least" affected rat were chosen from the four of each sex and strain in each replication principally on the basis of the ratio of activity after ethanol to that after saline from 16 to 30 minutes after injection; animals with the lowest ratios were "most affected." However, animals with outlying activity scores after saline were excluded (Riley et al. 1976). The "least affected" (LA) albinos were mated with "least affected" hooded rats, and the "most affected" (MA) albinos were mated with "most affected" hooded rats in each replication. Thus, the F_1 (first replication) and F_1' (second replication) generations each consisted of two LA litters and two MA litters. One rat of each sex was chosen from each litter, and the nonsibling matings produced the F_2 and F_2' generations which were combined to produce F_3 rats. Four breeding pairs per line were established after combining the replications. Rats were weaaned at 21 to 28 days of age and were housed with same-sex littermates thereafter. Testing was generally carried out at about 70 days of age. In the F_8 generation, use of a male and a female from each litter was discontinued in favor of taking the four most extreme rats of each generation but avoiding brother-sister matings. Beginning in the F_6 generation, second litters (designated by a prime, e.g., F_6') were bred to increase the supply of subjects. (The details of the breeding procedure were described extensively by Riley et al. 1976.)

Results and Discussion

The progress of the breeding project using the ratio of activity after ethanol to that after saline as the phenotype (this measure normalizes changes in apparatus sensitivity over generations) is presented in figure 1. As can be seen from figure 1, the lines did not differ significantly until the F_5 generation. The response to selection

Figure 1. Progress of Selective Breeding for Ethanol Sensitviity in LA and MA Rats



The response to selection for ethanol sensitivity is indicated by alcohol:saline activity ratios over the first 13 generations of selection. The probability value for the line effect as determined by unweighted means analysis is presented for each generation.

Reproduced with the publisher's permission from Worsham, Riley, Anandam, Lister, Freed, and Lester (1977). Copyright 1977 by Plenum Press.

for increased sensitivity (i.e., MA) was obtained more rapidly than that for decreased sensitivity (LA). The breeding project appears to have produced bidirectional changes in sensitivity as indicated by alcohol:saline activity ratios, but a control line would be required to determine if this is, in fact, the case.

Critical to the hypothesis that LA and MA rats differ in CNS sensitivity to ethanol is the demonstration that no line difference exists in the disposition of ethanol. When Riley et al. (1977) measured blood ethanol levels determined 10 minutes after completion of activity testing, they found no line difference in four of five generations and a very small difference in eighth generation animals; however, it was LA rats who had higher blood ethanol levels. Worsham and Freed (1977) also found slightly higher blood ethanol levels in LA rats than in MA rats of the F_{12} generation. Bass and Lester (1980) determined whole-brain ethanol levels in 11 LA and 12 MA rats of the F_{17}' generation 20 minutes after 1.75 g ethanol/kg, i.p.

Mean brain ethanol concentrations were 2.50 ± 0.05 SEM (standard error of the mean) mg ethanol/g brain in LA rats and 2.58 ± 0.06 mg/g in MA rats. There was no line difference, no sex difference, and no line X sex interaction. It is, therefore, most unlikely that the apparent differential sensitivity of LA and MA rats from 16 to 30 minutes after i.p. ethanol is attributable to differences in the distribution of ethanol.

The LA and MA rats also do not differ in their rates of ethanol clearance, despite the finding that alcohol dehydrogenase activity is about 18 percent higher in LA rats (Lester et al. 1977).

Differential depression of locomotor activity by ethanol in LA and MA rats is not specific to the stabilimeter apparatus; similar effects have been reported in the open field and in the running wheel (Worsham, Freed, Lochry, and Riley 1977). Nor is differential depression of activity in the stabilimeter apparatus specific to the 1.5 g/kg dose used for selective breeding; the line difference is observed over the dose range of 0.75 to 2.25 g ethanol/kg (Worsham and Freed 1977). Moreover, MA rats are more sensitive than LA rats to the soporific effect of ethanol: Riley et al. (1977) reported twofold to threefold differences in ethanol-induced (2.5 to 3.0 g/kg, i.p.) "sleep time" in rats of the fifth, eighth, and ninth generations. A more modest (approximately 50 percent), but still significant ($p < 0.02$), difference was observed after 3.5 g/kg in F_{17}rats (Bass and Lester, in preparation).

The MA rats are also more sensitive than LA rats to the soporific effects and to the locomotor depressant effects of sodium pentobarbital (Riley et al. 1978), a barbiturate that is cross-tolerant with ethanol (Frankel et al. 1977). For MA rats, the mean duration of loss of the righting reflex after 18 mg pentobarbital/kg was about 7 times longer than that of LA rats (Riley et al. 1978). Unfortunately, neither rates of pentobarbital metabolism nor the dose-response relations in these lines are known. In contrast to this differential sensitivity of LA and MA rats, Erwin et al. (1976) found no line difference in sleep time after pentobarbital (60 mg/kg) in LS and SS mice. Siemens and Chan (1976) reported pentobarbital (50 mg/kg) sleep time of SS mice to be approximately twice that of LS mice, but there was no line difference in waking brain levels.

While LA and MA rats have been selectively bred for sensitivity to motoric effects of ethanol, they may also be differentially sensitive to alcohol's effects on sensory processes. Fifteenth generation LA and MA rats received 15 noncontingent shocks consisting of five subcutaneously delivered shocks at each of three intensities (0.5, 0.8, and 1.3

mA) in a random order on a variable time 60-second schedule. Testing began 20 minutes after i.p. administration of 0.0 (saline), 0.67, 1.33, or 2.0 g ethanol/kg. Dose was varied within each line using a Latin square design such that each rat received each dose in a random order. Amplitude of the startle response and the number of audible vocalizations were recorded as described by Bass et al. (1978). The MA rats were more sensitive than the LA rats to the reduction of startle amplitude: 1.33 g/kg produced a marked reduction which was not further reduced by 2.0 g/kg in MA rats, while 1.33 g/kg caused a smaller reduction in LA rats and 2.0 g/kg produced a further reduction. Further, 1.33 and 2.0 g ethanol/kg produced greater reductions in the incidence of vocalizations in MA than in LA rats (Friedman et al. 1980). These reductions are not attributable to motor effects because rats of both lines showed significantly greater startle amplitude and number of vocalizations after 2.0 g/kg at 2.5 mA shock than at 1.3 mA. The MA rats are also more impaired by ethanol (0.75 to 1.75 g/kg; Bass and Lester, in press) in jumping to a pedestal (Tullis et al. 1977) to escape 0.3 mA grid shock.

The LA and MA rats have been found to differ in several non-alcohol-related phenotypes. The MA rats are heavier at birth, are equally heavy at weaning, and are lighter than LA rats thereafter (Worsham, Riley, Ananham, Lister, Freed, and Lester 1977). It is unlikely that this difference is causally related to differential alcohol sensitivity. The LA rats are more active in the running wheel than are MA rats, and it was speculated that the response-produced feedback is more aversive to MA rats, perhaps due to greater vestibular sensitivity (Riley et al. 1977). Bass and Lester (1979) found that MA rats swim reliably faster than LA rats; this is not inconsistent with the hypothesis offered. As a consequence of breeding for alcohol's impairment of motor performance, the breeding project may have emphasized the vestibular component of ethanol sensitivity more than other aspects of intoxication. Bass and Lester (in press) found that MA rats do not perform as well as LA rats in jumping onto a descending pedestal (Tullis et al. 1977) to escape 0.3 mA footshock. However, MA rats showed greater overt startle response to intermittent shocks (Friedman et al. 1980).

While LA and MA rats differ on a variety of alcohol- and pentobarbital-related phenotypes, described above, the lines do not differ on every alcohol-related phenotype. The LA and MA rats did not differ in maximum impairment on the moving belt test, although LA rats recovered their predrug performance more rapidly (Lester et al. 1977). Eighth generation LA and MA rats did not differ in consumption of saccharin-sweetened ethanol solutions (5.6 to 22.4 percent w/v) when they were offered as a sole fluid, and ninth generation rats did not differ in consumption of 5.6 percent unsweetened ethanol solution in a choice versus water (Riley et al. 1977). The lines did not differ in degree of conditioned aversion induced by 1.5 g ethanol/kg when ethanol was paired with either a saccharin or a saline solution (Worsham, Riley, Ananham, Lister, Freed, and Lester 1977), but MA rats showed a more pronounced aversion to 0.75 mmoles lithium chloride/kg when it was paired with 5 percent w/v ethanol (Worsham, Riley, Ananham, Lister, Freed, and Lester 1977). It is not clear, however, whether the lines differ in sensitivity to this LiCl dose or in associability of gastrointestinal distress with ethanol, as has been found in inbred mouse strains (Nachman et al. 1971).

In light of the multiple effects of ethanol, it is not surprising that not every effect is highly correlated with every other effect. However, Bass and Lester (1979) reported a line difference in ethanol sensitivity seemingly at odds with others previously described: Ethanol produced greater impairment of swimming (measured as increase over predrug swim time) in LA rats than in MA rats. The lines did not, however, differ in impairment of swimming by a range of sodium pentobarbital doses.

Line differences in initial sensitivity to ethanol are maintained after acquisition of tolerance. The MA rats were still more sensitive than the LA rats to the locomotor depressant and soporific effects of ethanol after tolerance was produced by intubation with 4.0 g ethanol/kg on alternate days for 3 weeks (Riley and Lochry 1977). Likewise, LA rats were still more sensitive than MA rats to impairment of swimming after intubation with ethanol increasing from 3.5 to 6.5 g ethanol/kg/day; there was no line difference in rates of tolerance acquisition or loss (Bass and Lester 1980). A parallel finding was reported by Nikander and Pekkanen (1977): After a 19-day tolerance-inducing chronic treatment regimen, alcohol-preferring AA rats (Eriksson 1971) remained less impaired on the tilting plane after ethanol treatment than alcohol-avoiding ANA rats.

Ethanol-induced impairment of swimming and depression of locomotor activity are dose dependent over the same range of doses in randomly bred rats (Bass and Lester 1978; Riley et al. 1976) and in LA and MA rats (Bass and Lester 1979; Worsham and Freed 1977). Task-dependent genotypic variations in ethanol sensitivity over a common dose range point to the nonunitary nature of ethanol-induced behavioral depression. Different tasks are likely to produce different patterns of neuronal activation. The effect of ethanol on a task depends on ethanol's effects on those patterns of activation occasioned by the task; therefore, neurochemical effects of ethanol are likely to vary with the behavioral situation. We suggest that at least some of the apparent conflicts in the literature on neurochemical effects of ethanol (Blum et al. 1977) may be attributable to often unreported experimental details, such as whether the treatment determination interval is spent in the home cage or in a novel environment, or whether the animal is held alone or in a groupp.

Task-dependent line differences in ethanol sensitivity indicate that there is no generalizable relation between sensitivity to acute motor effects and sensitivity to chronic effects of ethanol. Disparate conclusions would be reached depending on which acute (and probably which chronic) effects are evaluated.

The integrity of line differences in sensitivity after development of tolerance, regardless of direction of sensitivity, and the lack of difference in the kinetics of acquisition or loss of tolerance in animals differentially sensitive to ethanol intoxication imply orthogonality between mechanisms mediating sensitivity and tolerance. Other findings (e.g., Frankel et al. 1975; LeBlanc et al. 1976; Ritzmann and Tabakoff 1976) appear to be consistent with this notion.

The greater sensitivity of MA rats to locomotor depressant and soporific effects of pentobarbital (Riley et al. 1978) and the lack of a line difference in impairment of swimming by pentobarbital (Bass and Lester 1979) indicate that a distinction between cross-sensitivity and cross-tolerance must be made. Just as ethanol sensitivity shows task-dependent variations (Bass and Lester 1979), cross-sensitivity between ethanol and barbiturates is task dependent. We would expect that chronic treatment with ethanol or pentobarbital would produce cross-tolerance to impairment of swimming by the other drug. However, it is not known to what extent the expression of cross-tolerance would depend upon the test situation.

The existence of task-dependent variations in ethanol sensitivity in rats selectively bred for this phenotype raises questions about the relationship among various indexes of sensitivity to the effects of ethanol. Selective breeding is a tool to reduce the probability of spurious correlations between traits, as may be found in inbred strains (Deitrich and Collins 1977), and in some sense it would be surprising if such closely related phenotypes (indexes of sensitivity to the same doses of the same drug) are totally unrelated. However, spurious associations between traits should not be ruled out, especially in view of the small number of breeding pairs per generation and the absence of replicate lines. Whether the reversed order of sensitivity of LA and MA rats on the swim task versus other effects is attributable to pleiotropic effects of those genes underlying other line differences in sensitivity or is independent of these other effects is a question we hope to answer.

Finally, the finding of task-dependent variations in ethanol sensitivity raises questions about the mechanisms underlying the various line differences in response to ethanol (described above) which go in the expected direction (i.e., MA rats showing more sensitivity). Had the line difference in impairment of swimming by ethanol been in the same direction as the others described, we might have assumed a common basis for all of these effects. It can now be asked whether those other effects have a common basis.

References

Bass, M.B., and Lester, D. Swimming as a measure of motor impairment after ethanol and pentobarbital in rats. *J Stud Alcohol*, 39:1618-1622, 1978.

Bass, M.B., and Lester, D. Rats bred for ethanol sensitivity: Impairment of swimming by ethanol and pentobarbital. *Psychopharmacology*, 63:161-167, 1979.

Bass, M.B., and Lester, D. Tolerance to ethanol-induced impairment of swimming in rats bred for ethanol sensitivity. *Psychopharmacology*, 71:153-158, 1980.

Bass, M.B., and Lester, D. Task-dependence ethanol effects on escape in rats bred for ethanol sensitivity. *Pharmacol Biochem Behav*, in press.

Bass, M.B.; Friedman, H.J.; and Lester, D. Antagonism of naloxone hyperalgesia by ethanol. *Life Sci*, 22:1939-1946, 1978.

Blum, K.; Hamilton, M.G.; and Wallace, J.E. Alcohol and opiates: A review of common neurochemical mechanisms. In: Blum, K., ed. *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms*. New York: Academic Press, 1977. pp. 203-236.

Dietrich, R.A., and Collins, A.C. Pharmacogenetics of alcoholism. In: Blum, K., ed. *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms*. New York: Academic Press, 1977. pp. 109-139.

Eriksson, K. Rat strains specially selected for their voluntary alcohol consumption. *Ann Med Exp Biol Fenn*, 49:68-72, 1971.

Erwin, V.G.; Heston, W.D.W.; McClearn, G.E.; and Deitrich, R.A. Effect of hypnotics on mice genetically selected for sensitivity to ethanol. *Pharmacol Biochem Behav*, 4:679-683, 1976.

Frankel, D.; Khanna, J.M.; LeBlanc, A.E.; and Kalant, H. Effect of p-chlorophenylalanine on the acquisition of tolerance to ethanol and pentobarbital. *Psychopharmacologia*, 44:247-252, 1975.

Frankel, D.; Khanna, J.M.; LeBlanc, A.E.; and Kalant, H. Effect of parachlorophenylalanine on development of cross tolerance between pentobarbital and ethanol. *Can J Physiol Pharmacol*, 55:954-957, 1977.

Friedman, H.J.; Bass, M.B.; and Lester, D. Ethanol-induced analgesia in rats selectively bred for ethanol sensitivity. *Pharmacol Biochem Behav*, 13(6):773-776, 1980.

LeBlanc, A.E.; Matsunga, M.; and Kalant, H. Effects of frontal polar cortical ablation and cycloheximide on ethanol tolerance in rats. *Pharmacol Biochem Behav*, 4:175-179, 1976.

Lester, D.; Lin, G.W-J.; Anandam, N.; Riley, E.P.; Worsham, E.D.; and Freed, E.X. Selective breeding of rats for differences in reactivity to alcohol: An approach to an animal model of alcoholism. IV. Some behavioral and chemical measures. In: Thurman, R.G.; Williamson, J.R.; Drott, H.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems. Vol. III. Intermediary Metabolism and Neurochemistry.* New York: Academic Press, 1977. pp. 635-642.

McClearn, G.E., and Kakihana, R. Selective breeding for ethanol sensitivity in mice. (Abstr.) *Behav Genet*, 3:409-410, 1973.

Nachman, M.; Larue, C.; and LeMagnen, J. The role of olfactory and orosensory factors in the alcohol preference of inbred strains of mice. *Physiol Behav*, 6:53-59, 1971.

Nikander, P., and Pekkanen, L. An inborn alcohol tolerance in alcohol-preferring rats. The lack of relationship between tolerance to ethanol and the brain microsomal ($Na_{+}K_{+}$) ATPase activity. *Psychopharmacology*, 51:219-223, 1977.

Riley, E.P., and Lochry, E.A. Effects of initial tolerance on acquired tolerance to alcohol in two selectively bred rat strains. *Drug Alcohol Depend*, 2:485-494, 1977.

Riley, E.P.; Freed, E.X.; and Lester, D. Selective breeding of rats for differences in reactivity to alcohol: An approach to an animal model of alcoholism. I. General procedures. *J Stud Alcohol*, 37:1535-1547, 1976.

Riley, E.P.; Lochry, E.A.; and Freed, E.X. Differential tolerance to pentobarbital in rats bred for differences in alcohol sensitivity. *Psychopharmacology*, 58:167-170, 1978.

Riley, E.P.; Worsham, E.D.; Lester, D.; and Freed, E.X. Selective breeding of rats for differences in reactivity to alcohol: An approach to an animal model of alcoholism. II. Behavioral measures in MA and LA rats. *J Stud Alcohol*, 38:1705-1717, 1977.

Ritzmann, R.F., and Tabakoff, B. Dissociation of alcohol tolerance and dependence. *Nature*, 263:418-420, 1976.

Siemens, A.J., and Chan, A.W.K. Differential effects of pentobarbital and ethanol in mice. *Life Sci*, 19:581-589, 1976.

Tullis, K.V.; Sargent, W.Q.; Simpson, J.R.; and Beard, J.D. An animal model for the measurement of acute tolerance to ethanol. *Life Sci*, 20:875-882, 1977.

Worsham, E.D., and Freed, E.X. Dose-response effects of alcohol upon rat strains bred for differences in reactivity to alcohol. *Pharmacol Biochem Behav*, 7:421-424, 1977.

Worsham, E.D.; Freed, E.X.; Lochry, E.A.; and Riley, E.P. Generality of differential sensitivity to alcohol in selectively bred rats. *Physio Psychol*, 5:429-432, 1977.

Worsham, E.D.; Riley, E.P.; Anandam, N.; Lister, P.; Freed, E.X.; and Lester, D. Selective breeding of rats for differences in reactivity to alcohol: An approach to an animal model of alcoholism. III. Some physical and behavioral measures. In: Gross, M.M., ed. *Alcohol Intoxication and Withdrawal.* Vol. IIIA. (*Vol. 85a of Advances in Experimental Medicine and Biology.*) New York: Plenum Press, 1977. pp. 71-81.

SECTION III: PHARMACOGENETIC PHENOTYPES

Introduction

Gerald E. McClearn

The field of pharmacogenetics consists of more than selective breeding studies. In this section, Horowitz provides a representative picture of the types of research in this extensive literature, emphasizing the utilization of inbred strains and, for the purpose of conserving space, restricting attention to opiate research. His review further exemplifies the ubiquity of genetic influence on drug-related characteristics. The plain fact seems to be that we could probably find strain differences or selectively breed for almost any trait of pharmacological interest.

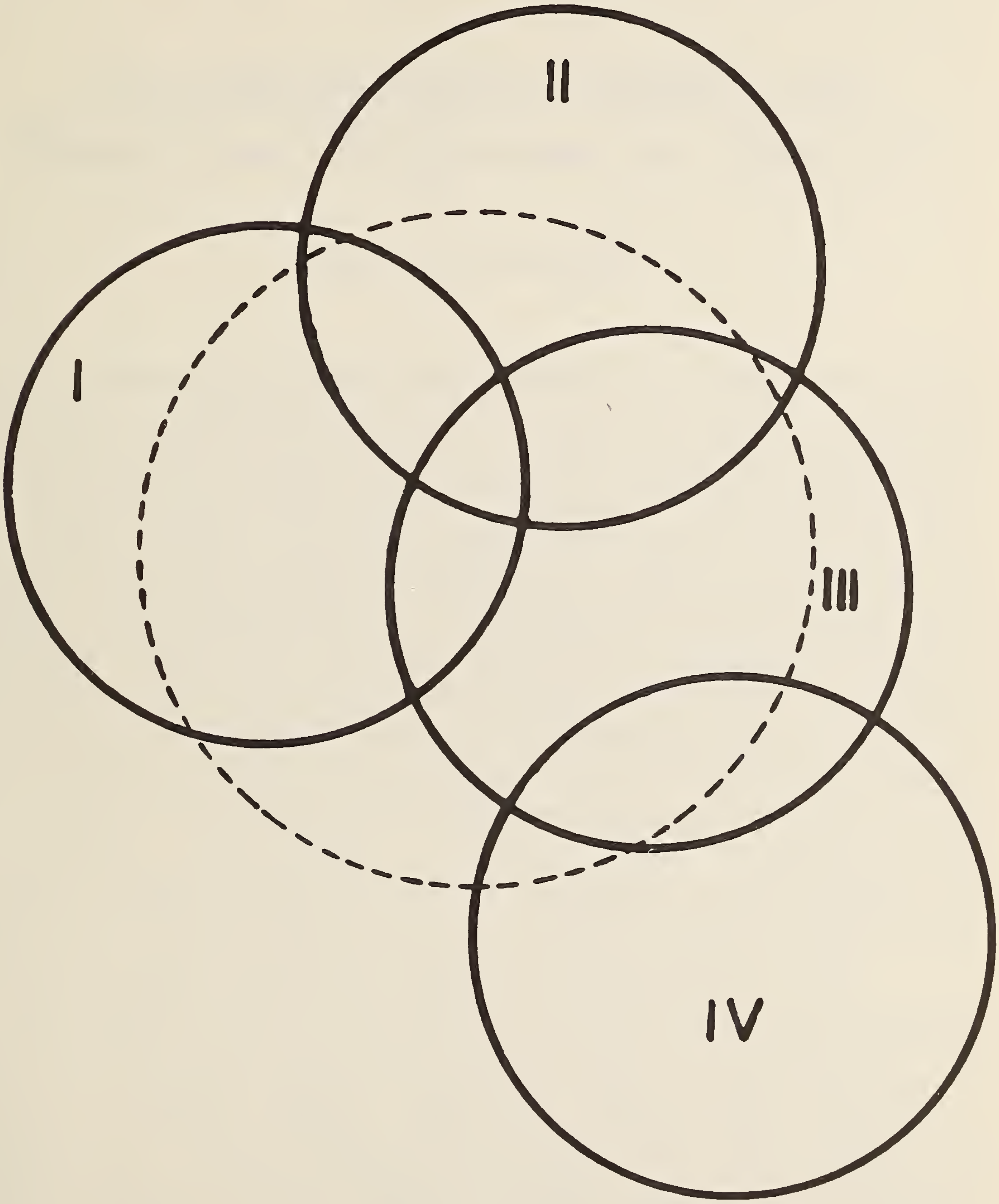
An underlying theme of the Horowitz presentation is that different procedures employed in defining a pharmacological parameter may give different results. For example, the tail flick test and the hot plate test used in defining opiate-induced analgesia provide dramatically different orderings of inbred strains.

Goldstein provides general considerations from the pharmacologist's point of view of the characteristics of desirable phenotypes and of suitable techniques of measurement in the context of selective breeding programs for alcohol research. She then explores possible phenotypes within the domains of ethanol metabolism, acute intoxication, tolerance, and physical dependence. With respect to the last-named domain, Goldstein identifies the same sort of problem discussed by Horowitz—the lack of a one-to-one correspondence between the index and the pharmacological process being indexed. The principal problem seems to be that there is no *unique* way to measure pharmacological processes such as analgesia, dependence, and tolerance. We employ indirect indexes of them such as tail flick latency or convulsion severity. These, of course, constitute operational definitions of the pharmacological parameter under investigation. But a priori there is no way of telling whether one index is "better" than another in some ultimate sense. We thus have analgesia$_1$, analgesia$_2$, dependence$_1$, dependence$_2$, etc.

This issue has relevance to selective breeding programs in at least two ways. First, we might initiate selective breeding using our favorite operational measure and study other indexes as correlated responses. Second, because selection is expensive and represents a major commitment of a laboratory, one might consider using information from more than one of the indexes to define the phenotype. Let us imagine, for example, that we have been allowed a cosmic glimpse of the "truth" and can identify all of the loci that have an influence on the real, true essence of the phenomenon of alcohol dependence. We might represent this real dependence by the dashed circle in figure 1. Now, any one of our ways of measuring dependence, such as the Goldstein-Pal procedure, may sample only some aspects of the phenomenon, shown by the circle labeled I. Other procedures, II-IV, measure other aspects. To the extent that these circles overlap we have an explanation of why different operational measures may agree in part. The extent of nonoverlap explains why we do not achieve identical results. We see also that all measures, to varying degrees, are measuring things not part of dependence. None of the measures shown exhaustively measures all the elements of dependence. A simple approach to the situation would be to combine measures by simply including all of them. However, from our cosmic perspective we can see that this approach is inefficient: All measures are not equally good. There is little point in using both measures III and IV because they themselves overlap so extensively in their sampling of real dependence. What we require is a system for providing differential weights that will give a new composite score that efficiently characterizes the phenomenon.

This specific problem is part of the general field of multivariate analysis. This is a richly articulated domain with diverse procedures for diverse problems. Meredith's chapter provides an introduction to this domain, with a special emphasis on some procedures that appear likely to be consequential for pharmacological research. It is probable that these multiple variable statistics will play an increasing role in future pharmacological research in general. In the new wave of selective breeding that we contemplate, multivariate considerations should not only permit us to generate better models but might also provide us with new insights into the very nature of the pharmacological processes themselves.

Figure 1. Hypothetical Representation of Alcohol Dependence and Its Measurement



The dashed circle represents the "real" elements of alcohol dependence. Various operational measures of dependence are represented by the circles labeled I through IV.

Pharmacogenetic Models and Behavioral Responses to Opiates*

Gary P. Horowitz

It is axiomatic that behavioral responses to drugs should not be different in principle from other forms of behavior and that behavioral traits in general are not different from physiological or morphological traits (Mayr 1970; Washburn and Shirek 1967; Whitney 1976). Therefore, individual differences in behavioral and physiological responses to pharmacologically active agents, such as drugs of abuse, would be expected to be influenced by genetic differences among the subjects in which the responses are measured. Pharmacogenetics is the emerging discipline concerned with the relative contribution of genetic factors to individual differences in responses to drugs. As a subset of this discipline, behavioral pharmacogenetics is concerned with those responses that are behavioral in nature. As its cumbersome name implies, it is the merging of elements with common interest from the fields of psychology, pharmacology, and genetics, passing through the hybrid stages of psychopharmacology, behavioral genetics, and pharmacogenetics (Eleftheriou 1975).

The emerging discipline is still in its infancy, but as a good hybrid should, it has shown incredible heterosis. There is a beauty in this fact for those actively engaged in research within the field. However, it creates a difficult problem when one is faced with presenting a complete but concise review of progress in the area. Though the present review may be concise, it cannot help but be incomplete.

---

*The research presented in this review was supported in part by grants DA-00828 and MH-11218 while the author was a doctoral student at Florida State University. Other aspects of the research were supported by a training agent, DA-07043, awarded to Richard A. Deitrich, University of Colorado Health Sciences Center. Portions of these data have previously been presented at meetings of the Behavior Genetics Association.

Fortunately, more extensive reviews are available (Broadhurst 1978; Eleftheriou 1975).

The present review is concerned primarily with the pharmacogenetics of individual differences in behavioral responses to opiates. It is important to note, however, that the successful research strategies applied to the study of alcohol and presented by other contributors to this volume can be applied to all drugs of interest. Similarly, it is hoped that a review of research strategies employed in the investigation of the pharmacogenetic aspects of responses to opiates will be of interest to researchers in other areas of pharmacology and pharmacogenetic research. The exclusive concentration on opiates should not be construed as a value judgment on the relative merits of studying any one of the number of potential drugs of interest. Rather, it is hoped that the review of opiates will provide a framework of organization that might apply to the study of any drug of choice.

Current interest in opiates stems from at least three main sources. First, opiates and opioid drugs remain as first-line drugs of choice in the medical treatment of severe pain (Jaffe 1970). Morphine, a naturally occurring opiate, is the standard against which new drugs are measured for analgesic potency. In addition, the continued recreational use and abuse of these compounds (notably, heroin) remain a major medical and sociological problem. Finally, the relatively recent discovery of endogenous polypeptides that have pharmacological and behavioral properties remarkably similar to traditional opiates (Snyder 1977) understandably has generated a new enthusiasm for elucidating the factors influencing responses to opiates.

As with any phenomenon of interest to behavioral genetics, a pharmacological approach can prove useful only if quantifiable variability in a given response can be demonstrated within a given species (Eleftheriou and Elias 1975). Intimations of individual differences with respect to responses to opiates are evident in human populations in both their medical and recreational applications. For example, Lasagna and Beecher (1954) reported that the "optimal dose" of morphine (10 mg/70 kg body weight) did not relieve severe pain in 33 percent of the patients that they surveyed in a clinical situation. Increasing the dose by 5 mg/70 kg still left 20 percent of the patients inadequately relieved from pain. Similarly, Wolff et al. (1966) reported significant intersubject differences among humans in responses to pain-inducing stimuli following morphine administration.

Individual differences among humans in liability to opiate abuse are also becoming apparent. The popular view that opiate use invariably leads to opiate dependence, and that opiate dependence is relatively permanent, has guided much of our public opinion for many years. However, surveys of opiate use by service personnel returning from Vietnam (Robins et al. 1974) have suggested that continued opiate use was rare and depended on a constellation of interacting factors. A recent report sponsored by the National Institute on Drug Abuse (O'Donnell et al. 1976) estimated that, among youthful heroin users who were interviewed, only about 10 percent were addicted to the drug.

The newest area of interest in opiates, that of endogenous opioid peptides, may also reveal individual differences in either the amount or efficacy of an internal analgesic system. For instance, naloxone (an opiate antagonist) has been shown to have differential effects on pain perception depending on whether the subject was reported to belong to a pain sensitive or pain insensitive subgroup (Buchsbaum et al. 1977). The differences in the effects of naloxone might be related to underlying differences in an endogenous opioid system.

Although the preceding paragraphs point to examples of individual differences among humans in responses to opiates, it is of course difficult to evaluate the contribution of genetic factors to these differences. Thus, it is fortunate that nonhuman analogs have been developed for the use of drugs in general (e.g., Amit and Sutherland 1975; Lester 1966) and opiates in particular (e.g., Nichols 1963; Stolerman and Kumar 1970). If we assume that responses to drugs in nonhuman subjects are different in a quantitative, rather than a qualitative, way from those of humans, then the experimental manipulation of genetic variables in studies using nonhuman subjects may help our understanding of the role of genetic influences on the etiology of individual differences in responses to drugs by humans. Therefore, the remainder of this review will be concerned with some relevant studies in the basic research on the pharmacogenetics of responses to opiates. Again, the studies selected for discussion are not an exhaustive list, but rather are chosen as representatives of a growing field of research.

Variability in responses to opiates has been demonstrated in mice and rats for both acute administration (e.g., Brase et al. 1977; Castellano and Oliverio 1975; Collins and Whitney 1978) and self-administration (e.g., Eriksson and Kiianmaa 1971; Horowitz et al. 1977; Satinder, 1977). Studies using each procedure are discussed separately, because the two procedures might be differentially relevant to a specific topic of human opiate use and abuse. That is, discrete administration procedures might be best used as analogs of individual differences in therapeutic uses of opiates, while self-administration procedures may prove more useful in providing models of opiate abuse and behavioral dependence. Furthermore, although the division is arbitrary except on a procedural basis, it does facilitate comparisons across individual studies.

Discrete Administration

For the most part, investigations of genetic factors influencing behavioral responses following acute administration of opiates (primarily morphine) have employed variations of two types of behavioral assays developed in nongenetic studies: a locomotor activity test (e.g., Dews 1953; Parker 1974) and/or an analgesic test with thermal stimulation using either a hot plate (e.g., Eddy and Leimbach 1953) or a tail flick (e.g., D'Amour and Smith 1941) method. Studies of genetic factors have included such tools of behavior genetic analysis as comparisons of inbred strains, recombinant inbred strains, genotypes derived for a classical genetic analysis, and, most recently, differentially selected lines of mice. These studies are in overall agreement in reporting genotypic differences in activity or analgesia, or both, following either single or repeated injections of morphine. The magnitude and direction of these strain differences are not always the same, even though some studies included mice of the same genotype. In some cases, differences in results might be attributable to differences in procedures, analyses, and behavioral assays, as well as to an interaction between these factors and underlying genetic differences.

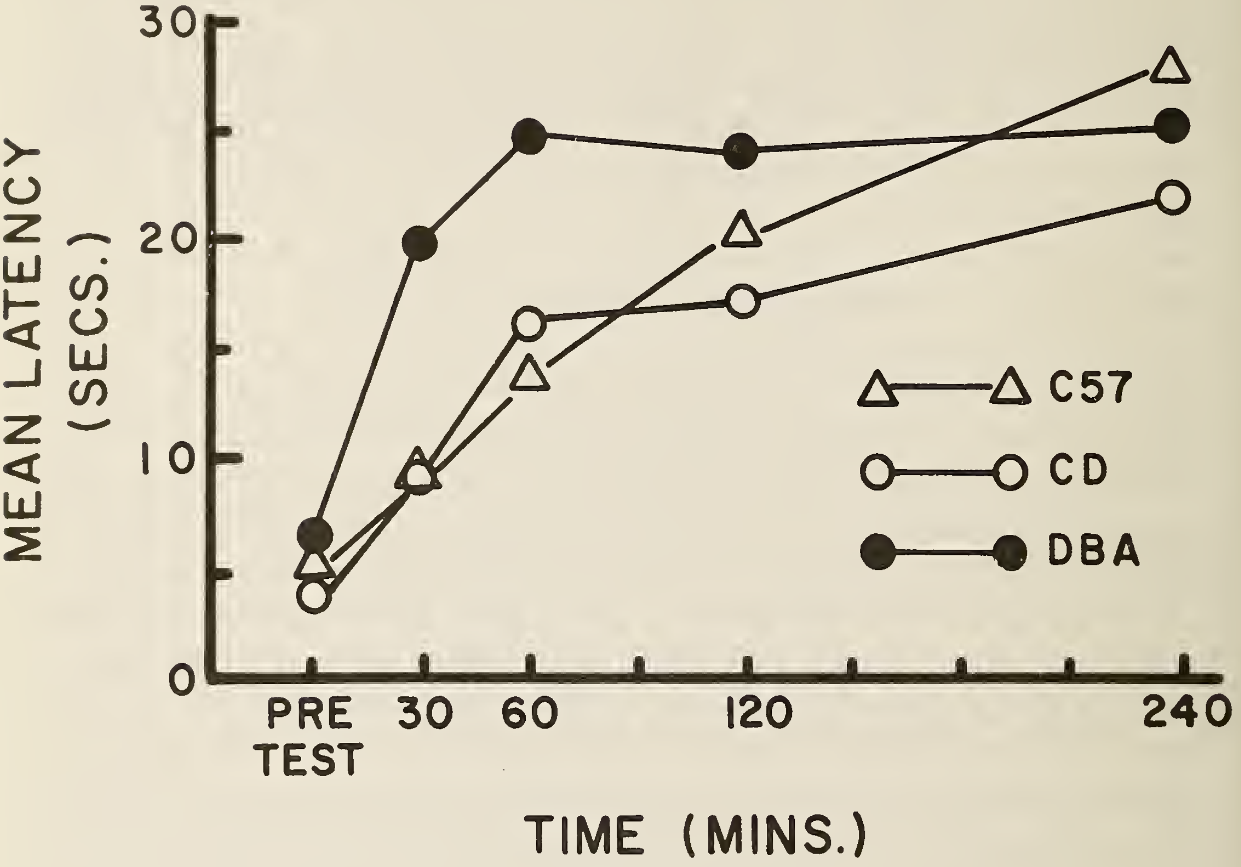
By way of illustration of the general procedures, consider the following study (Castellano and Oliverio 1975; Oliverio and Castellano 1974). In part, these researchers investigated morphine-induced locomotor activity and analgesia in adult male C57, BALB, and DBA inbred mice. Analgesia was measured on a hot plate. Each mouse was placed on a metal surface that was maintained at 58°C and covered with an inverted crystallizing dish. A trial was terminated when the mouse licked any paw, or when 30 seconds had elapsed without a lick. Mice of each genotype were tested on the hot plate prior to the administration of any drug, and a baseline latency to paw lick was recorded. The mice were then injected intraperitoneally with 5, 10, or

20 mg/kg of morphine and retested at 30, 60, 120, and 360 minutes postinjection. A schedule of repeated injections was followed in order to evaluate the development of tolerance in mice of each genotype, but only the results of the acute phase of the experiment are relevant to the present discussion.

Strain differences were reported in responses to thermal pain as measured by this method. There were no significant differences among mice of the three genotypes in latency to respond on the hot plate in the absence of the drug, with all strains showing a mean latency of about 10-12 seconds. However, 30 minutes following the first injection of morphine, differences in latency to respond were evident, and these differences were both dose and genotype dependent. For the 5 mg/kg dose groups, BALB mice showed the longest mean latency to respond (24.3 seconds), indicating the greatest sensitivity to the analgesic effects of morphine. At this dose, DBA mice showed an intermediate mean latency to respond (16.9 seconds), while C57 mice showed no evidence of morphine-induced analgesia (mean latency = 11.2 seconds). At the higher doses used by Oliverio and Castellano (1974), both DBA and BALB mice had the highest mean latencies possible (because subjects were removed from the hot plate if they failed to respond within 30 seconds). The C57 mice showed a linear increase in latency with increase in dose, reaching a mean latency of 23.9 seconds when tested 30 minutes following a 20 mg/kg injection of morphine. Thus, C57 mice appeared to be less sensitive to the analgesic effects of morphine than were DBA or BALB mice. The C57 mice also returned to predrug latencies sooner than did DBA or BALB mice.

Only a small portion of an excellent series of studies by these researchers and their associates has been presented above. However, the discussion allows the introduction of an important methodological consideration. It should be noted that a preinjection test was done for all subjects, and the preinjection latency was assumed to remain constant over subsequent postinjection tests. Changes across trials are attributed to the effects of the drug, when they may reflect changes in the baseline due to repeated trials. Figure 1 represents data gleaned from a study by Collins and Whitney (1978), which generally followed the procedure employed by Oliverio and Castellano (1974). The genotypes tested were C57, DBA, and CxD F_1hybrids. The latencies to respond on a hot plate are presented for each genotype for preinjection and 30-, 60-, 120-, and 240-minute postinjection trials. However, these animals were not injected with morphine, but rather with saline. Again, there were no differences among the

Figure 1. **Mean Latency to Respond to Hot Plate Stimulation Before and At Various Intervals After Saline Injection**



Adapted with the publisher's permission from Collins and Whitney (1978). Copyright 1978 by Springer-Verlag.

three genotypes in preinjection latencies, in agreement with the findings of Oliverio and Castellano. However, notice the pattern of responding in mice of the DBA strain. These mice increased in latency to paw lick by about 14 seconds from the pretest to the first postinjection test. This appears to be a drug effect, but the animals never received morphine. The salient points are that there are effects of repeated testing on the hot plate and that the magnitude of these effects are genotype dependent. Genetic differences in responses to thermal pain following morphine administration must be divorced as much as possible from differences among genotypes in response to the testing regimen in the absence of the drug.

Genetic factors influencing morphine-induced analgesia also seem to be affected by the task used to evaluate the contribution of these factors. Shuster (1975) investigated morphine-induced analgesia and activity in BALB/cBy, C57BL/6By, their reciprocal F_1 hybrids, and seven recombinant inbred strains derived from them. Briefly, recombinant inbred lines are derived from brother-sister matings of descendents of F_2 mice derived from the original BALB and C57 parental strains. These recombinant lines are inbred for several generations in order to maximize genetic homozygosity within each line, and each resulting line represents some recombination of the genes present in the parental strains. (For a fuller discussion of the development and use of recombinant inbred lines, see Bailey 1971.)

Shuster (1975) used the tail flick method of evaluating the analgesic effects of morphine, as developed by D'Amour and Smith (1941). This method measures the animal's latency to flick its tail when a beam of light of fixed intensity is focused on the tail. This latency was measured before, and at various times following, injections of either 2.5, 5, or 10 mg/kg of morphine.

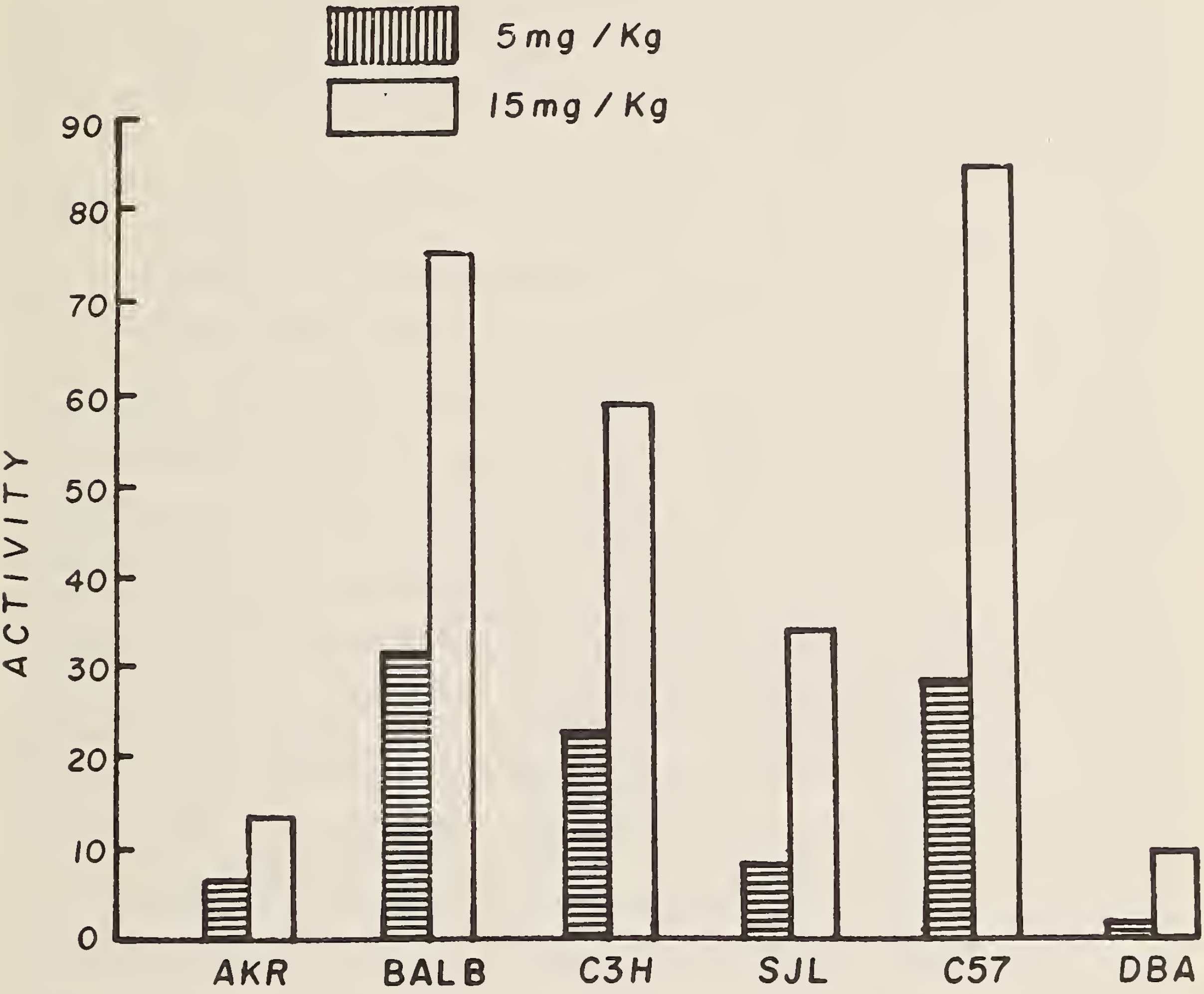
Two aspects of the results of this study are pertinent to the present discussion. First, strain differences existed in baseline responses to thermal pain as measured by this method. Second, when these differences in baseline response were taken into account, strain differences in changes in latency following all doses of morphine were also observed. Latencies to tail flick were longer for C57 mice than for BALB mice when measured 30 minutes after injection of any of the three doses of morphine used. The difference held across all postinjection tests. This finding is in contrast to that reported by other investigators. Oliverio and Castellano (1974) reported C57 mice to show less morphine-induced analgesia than BALB mice when analgesia was measured 30 minutes after the initial injection. Eidelberg et al. (1975) reported little difference between the two strains in the degree of hot plate analgesia 30 minutes after injection of either 5 or 10 mg/kg morphine, but BALB mice showed greater analgesia than C57 mice following injections of 20 mg/kg of the drug.

Shuster (1975) suggested that the nature of the two methods used to evaluate analgesic effects might explain why C57 mice displayed a higher degree of morphine-induced analgesia in his study, while BALB mice appeared to show more analgesia in those studies using the hot plate method. The hot plate method might be more influenced by the general activity of the subject (Vogt 1973). In the studies by Shuster (1975) and Oliverio and Castellano (1974), C57 mice were reported to be more active than BALB mice following morphine administration. Shuster suggested that the increased activity of C57 mice relative to the BALB strain may produce less of an analgesic effect "because the animal does not stay in one place long enough to feel the pain as soon as the mouse that remains stationary" (p. 76). If this is true, C57 mice can be expected to have higher latencies to respond on the hot plate relative to less active BALB mice. Such an effect was not evident in the study by Oliverio and Castellano (1974). Although this explanation of the mediation of test differences does not appear to be valid, the possibility that differences between the two procedures may influence the degree of analgesia exhibited by different strains of mice cannot be discounted.

Mice, in general, have been reported to respond to moderate doses of morphine with increased levels of locomotor activity (Goldstein and Sheehan 1969). Research by a number of different laboratories has shown that increased locomotor activity in response to opiates is species, genotype, and dose specific. The effects of genotype and dose of morphine were investigated in a study done in collaboration with Collins and Whitney (Horowitz, Collins, and Whitney 1977). The results of the study are summarized in figure 2. Six genotypes are represented. The dependent variable was activity counts (photobeam interruptions) in a circular chamber combined across postinjection trials. Each group has been corrected in a genotype-specific fashion for differences in baseline (no drug) activity across repeated trials. In general, dose differences are apparent, but the magnitude of these differences are genotype specific. In summary, it appears that C57, BALB, and C3H mice are most sensitive to the locomotor effects of morphine, SJL mice are intermediate, and DBA and AKR mice are only slightly affected. These results are in general agreement with those previously reported by Oliverio and Castellano (1974) and Brase et al. (1977).

If one chooses a different behavioral measure of morphine-induced activity, the clustering of genotypes with respect to degree of hyperactivity appears to change considerably. Eidelberg et al. (1975) investigated the influence of genetic factors on a number of behavioral assays following morphine injections. The subjects included mice from both the C57 and BALB strains, as well as two widely used noninbred lines, Swiss-Webster and ICR. Included in the behavioral assays was a test for morphine-induced running activity, which was measured in standard rotating running wheels. The results of this assay were different from those reported by Brase et al. (1977) or in the previously presented data from our laboratory. At 10 mg/kg, running activity was depressed for all genotypes tested, relative to their own individual baseline levels. In fact, Eidelberg et al. (1975) reported that C57 mice failed to increase their running activity following injections of any dose of morphine used in their study.

Figure 2. **Locomotor Activity of Six Inbred Mouse Strains After Intraperitoneal Injection of Morphine (5 mg/kg or 15 mg/kg)**



Scores for each strain have been combined across post injection trials and corrected for activity levels in the absence of the drug.

The lesson intended is similar to that following the discussion of hot plate analgesia. Broad statements regarding the effects of morphine on analgesia and activity are sometimes misleading because these statements are influenced by the behavioral assay used to measure each, by the number and spacing of drug-test pairings (Kayan et al. 1973; Siegel 1975, 1976, 1977), and by the response of the animal to the testing procedure in the absence of the drug (Collins and Whitney 1978). Furthermore, all these factors may interact with each other and with genetic factors, so that the evaluation of genetic factors influencing behavioral responses to morphine must take into account genotype by testing interactions in the absence of the drug. The problems associated with such an evaluation do not negate the contribution of the studies discussed in this review. Realization of the problems, however, does argue that statements and inferences about the results of these and other studies should be both task and procedure specific (Eleftheriou and Elias 1975). Overall statements regarding the influence of genetic factors on opiate-induced activity and analgesia per se must await either consensual validation from many studies or must come from studies designed to minimize these confounding interactions.

Differences in the effects of opiates on responses to painful stimuli and on locomotor activity are part of a larger question of the relationship between these two effects. The studies presented in this review have demonstrated that both opiate-induced activity and analgesia are influenced, at least to some extent, by genetic factors. It was suggested previously (Oliverio and Castellano 1974) that morphine-induced analgesia and activity are controlled by two different sites of action and may be under separate genetic control.

There is an increasing amount of behavioral, biochemical, phylogenetic, and anatomical evidence to support this suggestion. Opiate receptor sites have been found in all vertebrate species tested (Snyder 1977), while morphine-induced increases in activity seem to be limited to mice, cats, rabbits, and, in small doses, rats (Ayhan and Randrup 1973). In those species that display both opiate-induced activity and analgesia, it has been demonstrated that these effects can be separated to some extent by biochemical, neuroanatomical, and genetic procedures. Rethy et al. (1971) found that injections of several types of opiates increased the locomotor activity and decreased the brain catecholamine content of mice, while inactive analogs and isomer forms of the drugs had no effect on either measure. This effect of catecholamine depletion can be heightened with p-chlorophenylalanine, a drug that depletes brain serotonin. Cheney and Goldstein (1971) found that p-chlorophenylalanine caused depletion of serotonin in mouse brains to 34 percent of the normal level and also caused potentiation of the hyperactivity induced by injections of levorphanol. Potentiation could be blocked by treatment with 5-hydroxytryptophan, a precursor of serotonin, prior to levorphanol injections. Injections of p-chlorophenylalanine had no effect on levorphanol-induced analgesia. Castellano et al. (1975) reported that morphine-induced hyperactivity could be reduced in C57 mice by pretreatment with α-methyl-p-tyrosine (AMT), a drug that inhibits tyrosine hydroxylase and depletes norepinephrine and dopamine levels in the brain (Snyder 1976). Pretreatment with AMT did not affect the analgesic response to morphine in C57 or DBA mice. In addition, it was reported by Trabucchi et al. (1976) that morphine injections resulted in increased levels of striatal cAMP in C57, but not DBA, mice. On the basis of the results of these studies, it appears that opiate-induced locomotor activity is related to catecholaminergic and serotonergic factors and that differences in the neurochemistry of these systems among strains of mice might mediate differences observed among these strains in the effects of opiates on activity at the behavioral level. On the other hand, opiate-induced analgesia seems to depend in part on the cholinergic system (Domino and Wilson 1973). Castellano et al. (1975) also reported that septal lesions reduced morphine-induced analgesia in both C57 and DBA mice, but did not affect the running response in either strain. The septal region is a source of axons that form cholinergic synapses with neurons in the hippocampus (MacIntosh 1976). It has been suggested (Oliverio and Castellano 1975) from these data that the limbic system, with cholinergic input via the septal region, modulates morphine-induced analgesia, but not activity. As stated previously, this latter effect seems to depend on monoamine systems.

Genetic differences at the behavioral level have also been suggested as evidence of a dissociation between the effects of morphine on pain sensitivity and activity. Oliverio and Castellano (1974) reported high activity, but low analgesia, following morphine injections in C57 mice, while DBA mice showed the opposite pattern of responses. Shuster (1975) reported that morphine-induced activity and analgesia segregated independently to some extent in recombinant inbred lines. Finally, Eidelberg et al. (1975) found that the rank ordering of morphine-induced activity, analgesia, tolerance, and dependence among the strains tested was different for each measure.

All of the findings mentioned in the preceding paragraph were used by the authors to support the hypothesis that morphine-induced analgesia and activity are, to some extent, under separate genetic control. The biochemical and physiological evidence reviewed previously also seems to support this suggestion. The interpretation of these findings as supporting the genetic dissociation hypothesis should be tempered by a consideration of the problems of methodology and procedure discussed previously. Regarding the segregation of analgesia and activity into cholinergic and catecholaminergic or serotonergic mediated effects, it may be more important to consider the interaction of one system with another, or the ratio of neurotransmitters to each other, rather than ascribing effects to individual systems. The complexities of the interactions of centrally acting drugs with one or more of these systems makes it difficult in practice to vary any one system without also changing other systems (Gebhart and Mitchell 1973). Differences in strain ordering for analgesic tests and activity tests following morphine administration are interesting and important findings. However, differences in the ordering of strains from one analgesia measure to another (e.g., Oliverio and Castellano 1974; Shuster 1975) or from one activity test to another (e.g., Eidelberg et al. 1975; Oliverio and Castellano 1974) must also be considered.

Self-Administration

Historically, nonhuman studies designed to investigate opiate self-administration have taken two general forms. The first method requires the animal to emit some response in order to receive an intravenous infusion of the drug (e.g., Deneau et al. 1969; Thompson and Schuster 1964; Weeks 1962). Behavioral dependence is inferred from the animal's responding, which either initiates or maintains drug administration (Deneau et al. 1969). Unfortunately, the method requires a considerable investment of time and effort for surgical preparation, training, and the monitoring of drug-directed behaviors. The operant method has not been used in genetic studies of opiate addiction liability because genetic studies require the testing of a large number of subjects.

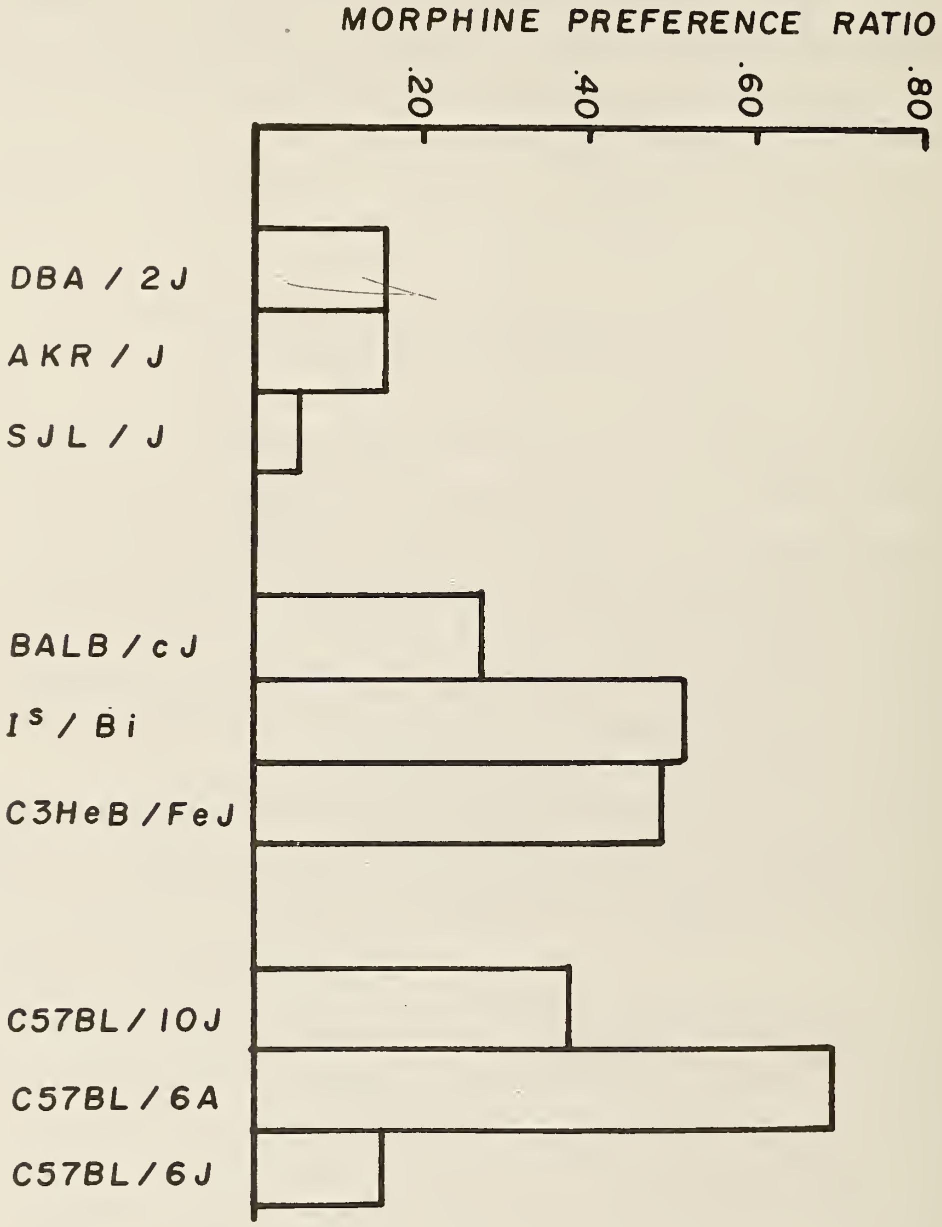
The second general method employed to induce self-administration of opiates by nonhuman subjects is to present the drug in the animal's food or drinking fluid (e.g., Cappell and LeBlanc 1971; Kumar et al. 1968; Shuster et al. 1963; Wikler et al. 1963). This approach has been fruitfully applied in genetic studies. One form of the procedure can be termed forced ingestion because the drug is presented in the animal's only source of food or fluid. For example, Meade et al. (1973) examined the amount of morphine consumption in two closed lines of rats (hooded and Wistar). Groups of rats from each line received morphine in water as their only available fluid for 5 days. The dependent measure was simply the amount of morphine solution ingested each day. The basic finding of the study was a line difference in that hooded rats drank consistently more morphine than did Wistar rats. The observed difference was not due to differences in previous water baseline, nor did the lines differ in their acceptance of another bitter solution, quinine.

Perhaps a more interesting variation of the oral ingestion procedures is to allow the animal a choice between a drug-adulterated solution and an alternative fluid. Here, the animal is never forced to consume the drug. Because morphine is bitter to humans, and presumably to nonhumans as well, it can be a difficult task to get the animals to drink the drug-adulterated solution in initial phases of these experiments.

The task is made somewhat simpler if the taste of the drug-adulterated solution is masked with a normally preferred substance, such as sucrose or saccharin. Experiments by Khavari et al. (1975) showed that rats prefer morphine in a sucrose vehicle to water in a two-bottle choice situation. These researchers demonstrated that rats could be made physically dependent on morphine and that a preference for morphine-adulterated solutions could be induced, even if the animals were never injected with the drug or forced to drink morphine-adulterated solutions to overcome thirst. During a 17-day period in which morphine was presented in a 10 percent sucrose vehicle in one tube and tap water in another, rats drank between 80 and 95 percent of their daily fluid intake from the sucrose-morphine tube. Subsequent challenge with naloxone indicated that the animals were also physically dependent on the drug.

The utility of being able to establish both behavioral and physical dependence on opiates in a relatively short period of time, and with no requisite of premedication or extensive training, is obvious, because the procedure is ideally suited for testing the large number of subjects required in genetic analyses. The success of the procedure, however, is very much dependent on the genetic conformation of the animals tested. By way of illustration, consider the following series of experiments (Horowitz, Collins, and Whitney 1977; Horowitz, Whitney, Smith, and Stephan 1977). They were designed to investigate the role of genetic factors influencing a predisposition to ingest morphine-adulterated solutions in a two-bottle free-choice situation, analogous to that described by Khavari et al. (1975). As previously stated, research in any area of pharmacogenetics is worthwhile only if divergent responses are evident within a given species. With regard to behavioral dependence, a second criterion is needed; subjects should be expected to select the drug-adulterated solution even when an alternative solution is available. Nine strains of mice were tested in a two-bottle choice situation, with one tube always containing tap water and the other tube containing either morphine, saccharin, or a morphine-saccharin mixture. The dependent variable was a preference ratio, which was calculated as the proportion of total fluid

Figure 3. Preference Ratios for Nine Mouse Strains When a Morphine Solution Was Offered Versus Water in a Two-Bottle Choice Situation



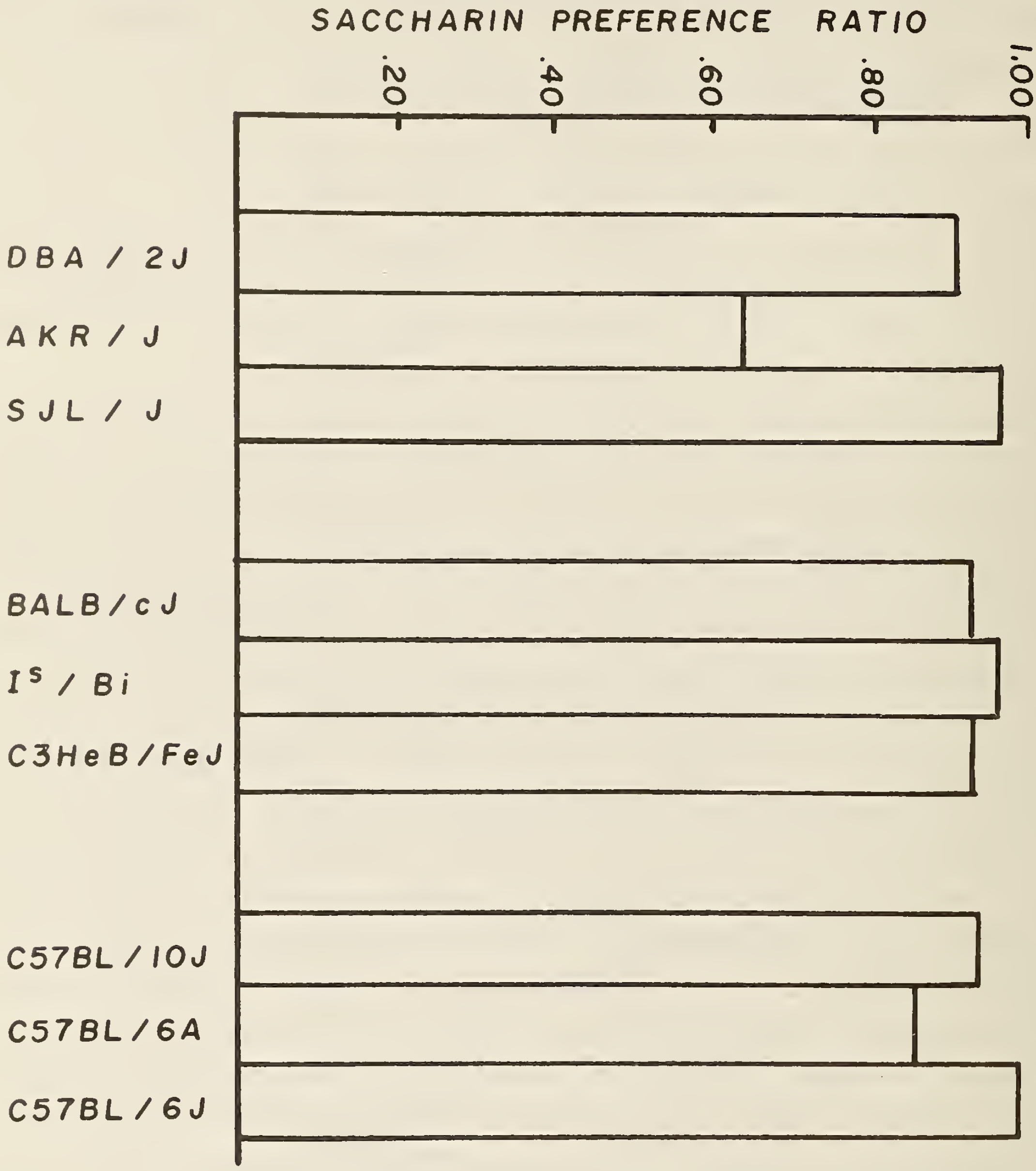
consumed in the form of the test solution. Figure 3 shows the results when the test solution was morphine (0.375 mg/ml of water). The criterion of variability is present to a certain degree, but only one strain (C57/6 Alberta) consistently selected morphine over water.

In accordance with the study conducted by Khavari et al. (1975), it was hoped that masking the presumably bitter taste of morphine would yield a higher level of drug ingestion. Saccharin (0.06 percent, w/v) was chosen as the masking vehicle. Figure 4 presents the mean preference ratios for the saccharin alone (no morphine) group for each of the nine genotypes. Mice of all strains preferred this concentration of saccharin over water, although AKR mice appeared to have a slightly lower preference than did mice of other genotypes. For mice in the third experimental group, the test solution was a morphine-saccharin mixture. The concentrations of saccharin (0.06 percent) and morphine (0.375 mg/ml) remained the same, but for mice in this group, the two agents were presented in the same drinking fluid. As is evident from figure 5, the results were very different from a simple additive effect. There was a wide and continuous range of variability in preference ratios, which varied from 0.04 to 0.98 (virtually from total avoidance to total preference). Absolute intake of the morphine-saccharin solution also showed considerable variability, ranging from 0.4 ml to 8.7 ml.

This initial strain survey was followed by a more detailed parametric analysis of genetic differences in ingestion of morphine-adulterated solutions. On the basis of the previous morphine-saccharin groups, a high-preferring genotype (C57BL/6J) and a low-preferring genotype (DBA/2J) were selected for subsequent investigation. In addition, both reciprocal F_1 genotypes were included. Mice of each genotype were tested under two-bottle choice situations using tap water versus an aqueous morphine solution (0.375 mg/ml), or various concentrations of saccharin, either alone or with morphine. The results of the experiment are presented in figure 6. As expected from the strain survey, none of the genotypes preferred morphine alone over water, as indicated by the square symbols in each panel. Similarly, mice of all genotypes preferred saccharin alone in concentrations ranging from 0.24 percent (w/v) on the first trial to 0.015 percent on the last trial (open circles in each panel). However, striking genotypic differences occurred in the morphine-saccharin groups. For C57 mice, there was no significant difference between preference for saccharin alone or morphine-saccharin when each solution was tested against water. For DBA mice, morphine-saccharin solutions were avoided as strongly as morphine solutions alone. There were no significant differences between reciprocal F_1 hybrids in preference for any test solution; for the combined F_1 genotypes, morphine-saccharin solutions were preferred over water, but were less preferred than saccharin alone. The mean morphine-saccharin preference displayed by F_1 hybrid mice was intermediate between that of the parental strains, but was directional toward the high-preferring C57 genotype.

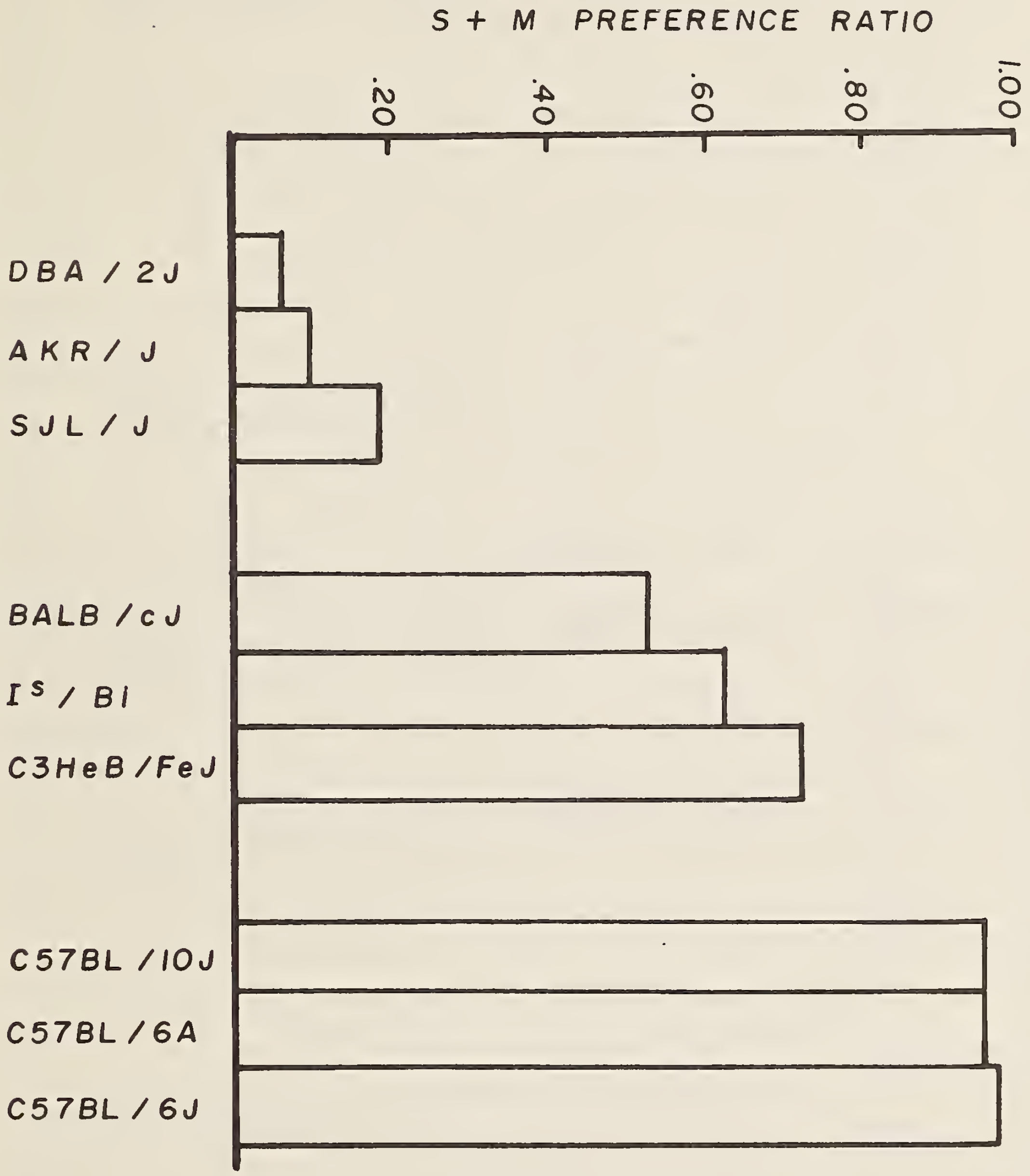
Although preference ratios reflect an important aspect of this experiment, differences in the absolute amount of morphine con-

Figure 4. Preference Ratios for Nine Mouse Strains When a Saccharin Soltuion Was Offered Versus Water in a Two-Bottle Choice Situation



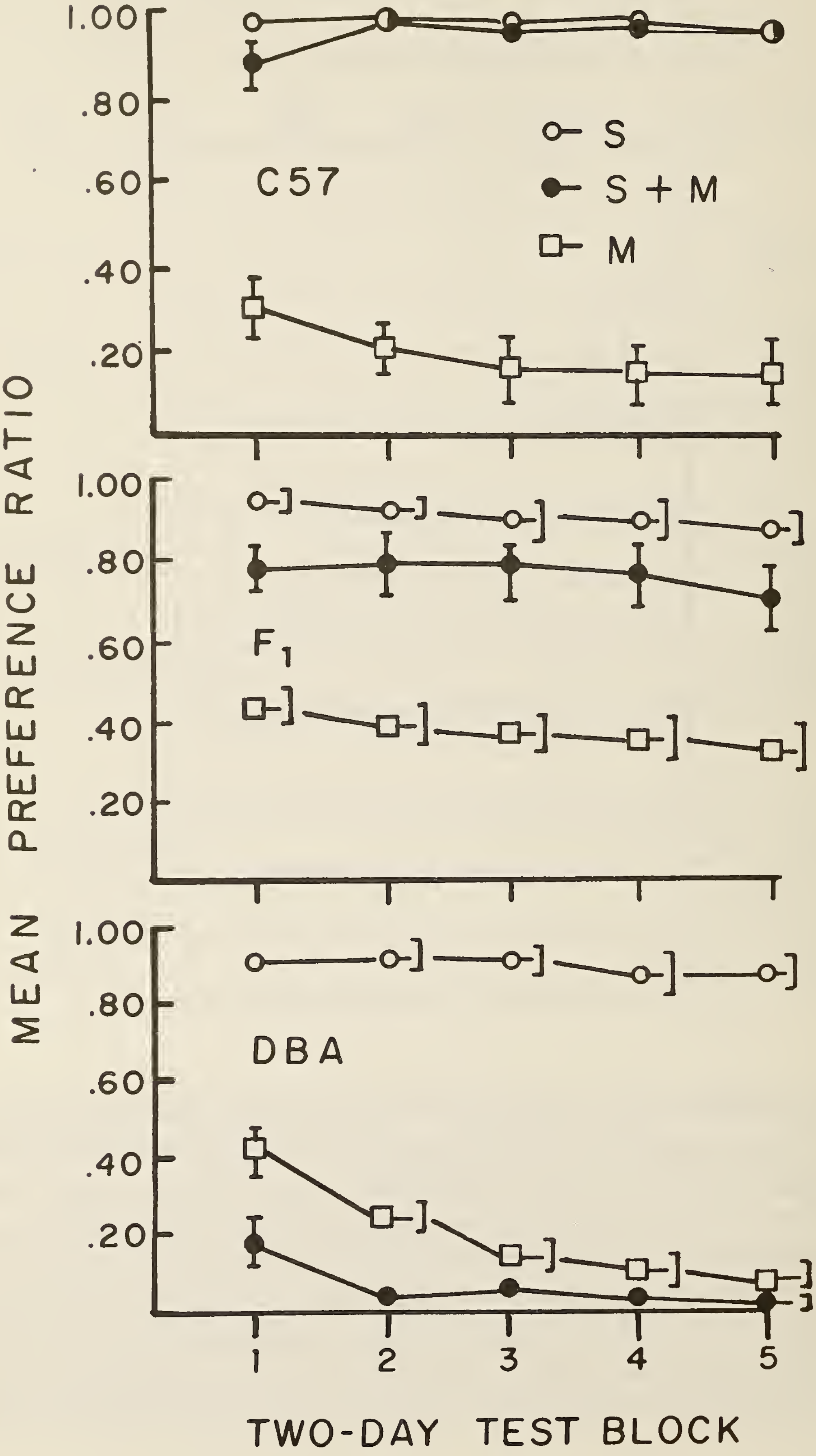
sumed by each genotype are equally relevant from a pharmacological viewpoint. In the morphine-saccharin condition, C57 mice ingested on the average an equivalent dose of 140 mg morphine/kg/day, even though they were never forced to drink the drug to overcome thirst. The mean daily dose for the reciprocal F_1 mice in this group was 67 mg/kg/day, while DBA mice consumed on the average less than 10 mg/kg/day under identical conditions. It should be remembered, of course, that this dose was distributed over the 12-hour dark phase of the photoperiod, when mice consume almost all of their daily fluid.

Figure 5. Preference Ratios for Nine Mouse Strains When a Morphine Saccharin Soltuion Was Offered Versus Water in a Two-Bottle Choice Situation



Similar differences in propensity to drink morphine-adulterated solutions were reported by Satinder (1977) for selected lines of rats. Two lines used in his experiment were selected for low (MNR/Har/Lu) and high (MR/Har/Lu) open-field emotionality, and there were also low (RLA/Lu), high (RHA/Lu), and unselected control (RCA/Lu) lines for rate of avoidance conditioning. Although the design was more complex than our experiment and involved interspersed forced trials, the results agreed with our finding that the degree of utility of masking the taste of morphine with a normally

Figure 6. Mean Daily Preference Ratios of C57, DBA, and F_1 Hybrid Mice for Morphine (M), Saccharin (S), or Morphine Saccharin (S + M) Solutions



preferred substance is genotype dependent. Three of the five genotypes tested developed a preference for morphine-adulterated solutions over water following a regimen of forced trials in which the drug was presented in a sucrose vehicle. It is interesting to note that those lines that developed a preference for morphine solutions over water (RHA, RLA, and MR) had been shown by Satinder (1976) to have relatively higher levels of emotionality in other behavioral tests.

In discussing oral ingestion of opiate-adulterated solutions, the present review has concentrated on those studies that were designed to test specifically the contribution of genetic factors to this behavioral phenotype. It should be noted, however, that individual differences in opiate ingestion have often been observed in studies not specifically designed to investigate genetic factors. For example, Khavari and Risner (1973) reported that one out of four rats offered a choice between morphine solutions and water drank considerably from the tube containing morphine. Similarly, using a forced ingestion procedure in rats, McMillan et al. (1976) reported three types of opiate drinkers. Some rats readily drank drug-adulterated solutions the first time these solutions were encountered. Other rats initially refused drug solutions, but began to drink significant amounts of the drug within 5 days. Finally, a significant number of rats refused to drink certain concentrations of morphine, methadone, or levorphanol during the first 5 days of exposure to these solutions. In fact, 2 out of 16 rats exposed to a 1 mg/ml solution of morphine refused to drink and died during the course of the experiment.

A conclusion that may be drawn with respect to opiate ingestion is analogous to that suggested regarding the effects of discrete administration. Statements about opiate ingestion must be tempered with descriptions of the subject population and by the vehicle and method of presentation of the drug-adulterated solution. It is readily apparent that individual differences in this form of drug-seeking behavior exist and that genetic factors are at least one source of variability.

Opiate-Alcohol Interactions

There are some ways in which an increased understanding of genetic contributions to individual differences in responses to opiates might be germane to the particular interests addressed in this monograph. Basic research using nonhuman subjects and surveys of human populations have yielded findings that suggest commonalities between the effects of, and responses to, both alcohol and opiates. Such commonalities have been suggested by studies of the biochemistry of alcohol metabolism and opiate synthesis (Davis and Walsh 1970), as well as by behavioral studies of the effects of administration of one of these agents on responses to chronic administration of the other drug (e.g., Ho et al. 1977). In addition, a general theory of addiction based in part on responses to opiates and alcohol, has been developed (Nichols 1972). A general review of the experimental protocols and results of these studies has been compiled by Blum et al. (1977). Opiate-alcohol commonalities might also be influenced by genetic factors. If such commonalities between the effects of opiates and alcohol are, in part, genetically mediated, then responses to these drugs might be expected to covary among genetically diverse subjects.

Nichols and Hsiao (1967) successfully bred rats for quantitative differences in readdiction susceptibility to morphine for three generations. Readdiction susceptibility was defined as the degree to which a rat would select morphine over water after it had previously been made dependent on the drug and then withdrawn. The rats were made dependent on morphine using a procedure of mixed forced and choice trials, accompanied by daily morphine injections. The dependence phase was followed by a period of 2 weeks during which the animal did not receive morphine in any form. This abstinence period was followed by a choice test between morphine and water, and morphine ingestion was used as an index of readdiction liability.

Following an initial screening test, rats were selected directionally for either high or low susceptibility to readdiction for three generations. The response to this selection produced both readdiction susceptible (S) and resistant (R) rats. The two lines did not differ on initial preference for morphine solutions or on quinine preference. However, naive subjects from the S_3 and R_3 selected lines differed in susceptibility to alcohol readdiction. Furthermore, the direction of the difference was the same as that for morphine.

Several other studies give less direct evidence for genetic mediation of opiate-alcohol interactions. A study similar to the experiment by Nichols and Hsiao (1967) was conducted by Eriksson and Kiianmaa (1971) using inbred strains of mice known to differ in initial alcohol preference and their derived generations. These latter researchers tested for morphine readdiction susceptibility, and genotypic differences observed for this phenotype closely paralleled differences previously reported for alcohol preferences. Satinder (1977) also reported that the genetic differences in his study of oral ingestion of morphine with selected lines of rats were similar to previous findings (Satinder 1972, 1975) for alcohol intake in these lines. Finally, a pilot study conducted in collaboration with Andrew Smolen and Allan Collins (Horowitz et al. 1978) suggested that the Colorado lines of mice selected for alcohol sleep time differed in response to lethal doses (LD) of morphine. Specifically, short-sleep mice showed a significantly lower LD_{50} following intraperitoneal injections of morphine than did long-sleep mice.

It should be realized that, even collectively, these data must be viewed as suggestive. As Satinder (1977) pointed out, the hypothesis of genetic mediation of opiate-alcohol interactions must be tested for phenotypic correlation among segregating populations. The hypothesis may also benefit greatly by accumulating evidence from selection studies of other alcohol and opiate phenotypes.

References

Amit, Z., and Sutherland, E.A. The relevance of recent animal studies for the development of treatment procedures for alcoholics. *Drug Alcohol Depend*, 1:3-13, 1975.

Ayhan, I.H., and Randrup, A. Behavioural and pharmacological studies on morphine-induced excitation of rats. Possible relation to brain catecholamines. *Psychopharmacologia*, 29:317-328, 1973.

Bailey, D.W. Recombinant inbred strains. *Transplantation*, 11:325-327, 1971.

Blum, K.; Hamilton, M.G.; and Wallace, J.E. Alcohol and opiates: A review of common neurochemical and behavioral mechanisms. In: Blum, K., ed. *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms.* New York: Academic Press, 1977.

Brase, D.A.; Loh, H.H.; and Way, E.L. Comparison of the effects of morphine on locomotor activity, analgesia and primary and protracted physical dependence in six mouse strains. *J Pharmacol Exp Ther*, 201:368-374, 1977.

Broadhurst, P.L. *Drugs and the Inheritance of Behavior.* New York: Plenum Press, 1978.

Buchsbaum, M.S.; Davis, G.C.; and Bunney, W.E., Jr. Naloxone alters pain perception and somatosensory potentials in normal subjects. *Nature*, 270:620-622, 1977.

Cappell, H., and LeBlanc, A.E. Some factors controlling oral morphine intake in rats. *Psychopharmacologia*, 21:192-201, 1971.

Castellano, C.; Espinet-Llovera, B.; and Oliverio, A. Morphine reduced running and analgesia in two strains of mice following septal lesions or modification of brain amines. *Naunyn-Schmiedebergs Arch Pharmacol*, 288:355-370, 1975.

Castellano, C., and Oliverio, A. A genetic analysis of morphine-induced running and analgesia in the mouse. *Psychopharmacologia*, 41:197-200, 1975.

Cheney, D.L., and Goldstein, A. The effect of p-chlorophenylalanine on opiate-induced running, analgesia, tolerance and physical dependence in mice. *J Pharmacol Exp Ther*, 177:309-315, 1971.

Collins, R.L., and Whitney, G. Genotype and test experience determine responsiveness to morphine. *Psychopharmacology*, 56:57-60, 1978.

D'Amour, F.E., and Smith, D.L. A method for determining loss of pain sensation. *J Pharmacol Exp Ther*, 72:74-79, 1941.

Davis, V.E., and Walsh, M.J. Alcohol, amines and alkaloids: A possible biochemical basis for alcohol addiction. *Science*, 167:1005-1007, 1970.

Deneau, G.; Yanagita, T.; and Seevers, M.H. Self-administration of psychoactive substances by the monkey. *Psychopharmacologia*, 16:30-48, 1969.

Dews, P.B. The measurement of the influence of drugs on voluntary activity in mice. *Br J Pharmacol*, 8:46-48, 1953.

Domino, E.F., and Wilson, A. Effects of narcotic analgesic agonists and antagonists on rat brain acetylcholine. *J Pharmacol Exp Ther*, 184:18-32, 1973.

Eddy, N.B., and Leimbach, D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *J Pharmacol Exp Ther*, 107:385-393, 1953.

Eidelberg, E.; Erspamer, R.; Kreinick, C.J.; and Harris, J. Genetically determined differences in the effects of morphine on mice. *Eur Pharmacol*, 32:329-336, 1975.

Eleftheriou, B.E., ed. *Psychopharmacogenetics*. New York: Plenum Press, 1975.

Eleftheriou, B.E., and Elias, P.K. Recombinant inbred strains: A novel approach for psychopharmacogeneticists. In: Eleftheriou, B.E., ed. *Psychopharmacogenetics*. New York: Plenum Press, 1975.

Eriksson, K., and Kiianmaa, K. Genetic analysis of susceptibility to morphine addiction in inbred mice. *Ann Med Exp Biol Fenn*, 49:73-78, 1971.

Gebhart, G.F., and Mitchell, C.L. Strain differences in the analgesic response to morphine as measured on the hot plate. *Arch Int Pharmacodyn Ther*, 201:128-140, 1973.

Goldstein, A., and Sheehan, P. Tolerance to opiod narcotics. I. Tolerance to the "running fit" caused by levorphanol in the mouse. *J Pharmacol Exp Ther*, 169:175-184, 1969.

Ho, A.K.S.; Chen, R.C.A.; and Morrison, J.M. Opiate-ethanol interaction studies. In: Blum, K., ed. *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms*. New York: Academic Press, 1977.

Horowitz, G.P.; Collins, R.L.; and Whitney, G. "Ingestion of and Response to Morphine Among Mice." Paper presented at the meeting of the Behavior Genetics Association, Louisville, Ky., 1977.

Horowitz, G.P.; Smolen, A.; and Collins, A.C. "Pilot study on mice selected for alcohol sleep time." Unpublished paper, Boulder, Colo., 1978.

Horowitz, G.P.; Whitney, G.; Smith, J.C.; and Stephan, F.K. Morphine ingestion: Genetic control in mice. *Psychopharmacology*, 52:119-122, 1977.

Jaffe, J.H. Narcotic analgesics. In: Goodman, L., and Gilman, A., eds. *The Pharmacological Basis of Therapeutics*. New York: Macmillan, 1970.

Kayan, S.; Ferguson, R.K.; and Mitchell, C.L. An investigation of pharmacologic and behavioral tolerance to morphine in rats. *J Pharmacol Exp Ther*, 185:300-306, 1973.

Khavari, K.A., and Risner, M.E. Opiate dependence produced by ad libitum drinking of morphine in water, saline and sucrose vehicles. *Psychopharmacologia*, 30:291-302, 1973.

Khavari, K.A.; Peters, T.C.; Baity, P.L.; and Wilson, A.S. Voluntary morphine ingestion, morphine dependence, and recovery from withdrawal signs. *Pharmacol Biochem Behav*, 3:1093-1096, 1975.

Kumar, R.; Steinberg, H.; and Stolerman, I.P. Inducing a preference for morphine in rats without premedication. *Nature*, 218:564-565, 1968.

Lasagna, L., and Beecher, H.K. The optimal dose of morphine. *J Am Med Assoc*, 156:230-234, 1954.

Lester, D. Self-selection of alcohol by animals, human variation, and the etiology of alcoholism: A critical review. *Q J Stud Alcohol*, 27:395-438, 1966.

MacIntosh, F.C. Acetylcholine. In: Siegel, G.J.; Albers, R.W.; Katzman, R.; and Agranoff, B.W., eds. *Basic Neurochemistry*. 2d ed. Boston: Little, Brown, 1976.

Mayr, E. *Population, Species and Evolution*. Cambridge, Mass.: Harvard University Press, 1970.

McMillan, D.E.; Leander, J.D.; Wilson, T.W.; Wallace, S.C.; Fix, T.; Redding, S.; and Turk, R.T. Oral ingestion of narcotic analgesics by rats. *J Pharmacol Exp Ther*, 196:269-279, 1976.

Meade, R.; Amit, Z.; Pachter, W.; and Corcoran, M.E. Differences in oral intake of morphine by two strains of rats. *Res Commun Chem Pathol Pharmacol*, 6:1105-1108, 1973.

Nichols, J.R. A procedure which produces sustained opiate-directed behavior (morphine addiction) in the rat. *Psychol Rep*, 13:895-904, 1963.

Nichols, J.R. Alcoholism and opiate addiction: Theory and evidence for a genetic link between the two. In: Forsander, O., and Eriksson, K., eds. *Biological Aspects of Alcohol Consumption*. Helsinki: Finnish Foundation for Alcohol Studies, 1972.

Nichols, J.R., and Hsiao, S. Addiction liability of albino rats: Breeding for quantitative differences in morphine drinking. *Science*, 157:561-563, 1967.

O'Donnell, J.A.; Voss, H.L.; Clayton, R.R.; Slatin, G.T.; and Room, R.G.W. *Young Men and Drugs—A Nationwide Survey*. NIDA Research Monograph No. 5. Rockville, Md.: National Institute on Drug Abuse, 1976.

Oliverio, A., and Castellano, C. Genotype-dependent sensitivity and tolerance to morphine and heroin: Dissociation between opiate induced running and analgesia in the mouse. *Psychopharmacologia*, 39:13-22, 1974.

Oliverio, A., and Castellano, C. Exploratory activity: Genetic analysis of its modification by various pharmacologic agents. In: Eleftheriou, B.E., ed. *Psychopharmacogenetics*. New York: Plenum Press, 1975.

Parker, R.B. Mouse locomotor activity: Effect of morphine, narcotic antagonists, and the interaction of morphine and narcotic antagonists. *Psychopharmacologia*, 38:15-23, 1974.

Rethy, C.R.; Smith, C.B.; and Villarreal, J.E. Effects of narcotic analgesics upon the locomotor activity and brain catecholamine content of the mouse. *J Pharmacol Exp Ther*, 176:472-479, 1971.

Robins, L.N.; Davis, D.H.; and Durco, D.N. How permanent was Vietnam drug addiction? *Am J Public Health*, 64(Suppl.):38-43, 1974.

Satinder, K.P. Behavior-genetic-dependent self-selection of alcohol in rats. *J Comp Physiol Psychol*, 80:422-434, 1972.

Satinder, K.P. Interactions of age, sex and long-term alcohol intake in selectively bred strains of rats. *J Stud Alcohol*, 36:1493-1507, 1975.

Satinder, K.P. Reactions of selectively bred strains of rats to a cat. *Anim Learn Behav*, 4:172-176, 1976.

Satinder, K.P. Oral intake of morphine in selectively bred rats. *Pharmacol Biochem Behav*, 7:43-49, 1977.

Shuster, L. Genetic analysis of morphine effects: Activity, analgesia, tolerance and sensitization. In: Eleftheriou, B.E., ed. *Psychopharmacogenetics*. New York: Plenum Press, 1975.

Shuster, L.; Hannam, R.V.; and Boyle, W.E., Jr. A simple method for producing tolerance to Dihydromorphinone in mice. *J Pharmacol Exp Ther*, 140:149-154, 1963.

Siegel, S. Evidence from rats that morphine tolerance is a learned response. *J Comp Physiol Psychol*, 89:498-506, 1975.

Siegel, S. Morphine analgesic tolerance: Its situation specificity supports a Pavlovian conditioning model. *Science*, 193:323-325, 1976.

Siegel, S. Morphine tolerance acquisition as an associative process. *J Exp Psychol: Anim Behav Proc*, 3:1-13, 1977.

Snyder, S.H. Catecholamines, serotonin, and histamine. In: Siegel, G.J.; Albers, R.W.; Katzman, R.; and Agranoff, B.W., eds. *Basic Neurochemistry*. 2d ed. Boston: Little, Brown, 1976.

Snyder, S.H. Opiate receptors and internal opiates. *Sci Amer*, 236(6):44-56, 1977.

Stolerman, I.P., and Kumar, R. Preference for morphine in rats: Validation of an experimental model of dependence. *Psychopharmacologia*, 17:135-150, 1970.

Thompson, T., and Schuster, C.R. Morphine self-administration, food-reinforced and avoidance behaviors in rhesus monkeys. *Psychopharmacologia*, 5:87-94, 1964.

Trabucchi, M.; Spano, P.F.; Racagni, G.; and Oliverio, A. Genotype-dependent sensitivity to morphine: Dopamine involvement in morphine-induced running in the mouse. *Brain Res*, 114:536-540, 1976.

Vogt, M. Types of neurones involved in the analgesic effect of morphine. In: Kosterlitz, H.W.; Collier, H.O.J.; and Villarreal, J.E., eds. *Agonist and Antagonist Actions of Narcotic Analgesic Drugs*. Baltimore, Md.: University Park Press, 1973.

Washburn, S.L., and Shirek, J. Human evolution. In: Hirsch, J., ed. *Behavior-Genetic Analysis*. New York: McGraw-Hill, 1967.

Weeks, J.R. Experimental morphine addiction: Method for automatic intravenous injections in unrestrained rats. *Science*, 138:143-144, 1962.

Whitney, G. Genetic considerations in studies of the evolution of the nervous system and behavior. In: Masterton, R.B.; Hodos, W.; and Jerison, H., eds. *Evolution, Brain and Behavior: Persistent Problems*. Hillsdale, N.J.: Erlbaum, 1976.

Wikler, A.; Martin W.R.; Pescor, F.T.; and Eades, C.G. Factors regulating oral consumption of an opioid (etonitazene) by morphine-addicted rats. *Psychopharmacologia*, 5:55-76, 1963.

Wolff, B.B.; Kantor, T.G.; Jarvik, M.E.; and Laska, E. Response of experimental pain to analgesic drugs. I. Morphine, aspirin and placebo. *Clin Pharmacol Ther*, 7:224-238, 1966.

What Traits To Breed For and How To Measure Them*

Dora B. Goldstein

This chapter attempts to provide the viewpoint of a pharmacologist on two questions: (1) What characteristics of mice or rats might usefully be augmented or diminished to provide particularly apt subjects for alcohol research? (2) What tests should be used to monitor these traits as they progress from generation to generation? First, we will consider some general characteristics of desirable phenotypes for alcohol research. The proposed breeding project seems likely to be an expensive undertaking, so we will want to develop mice of the greatest possible usefulness. This means selecting phenotypes that represent areas of active ongoing research, the major themes of the past several years. Another valuable characteristic of the selected lines to be derived would be applicability to a variety of problems. These animals should be usable for multiple questions and in different types of laboratories. If it is feasible, it would be an advantage to have animals that can be used for very general pharmacological studies, not necessarily limited to alcohol. A line bred for susceptibility to seizures would be an example.

What kinds of techniques could be used to test the animals? The first necessity, of course, is that the testing procedure should not impair the animals' subsequent ability to breed. For a start, it must not be lethal. We are limited to studies of the whole animal, which could be physiological or behavioral, or to examination of accessible tissues such as blood, urine, expired air, or (possibly) biopsy specimens. We can examine animals in their normal state or after exposure to drugs (not limited to ethanol) or to various environmental conditions. Because individual animals must be scored or ranked, the measure must be graded, not quantal. In some respects, these tests need not be very elegant. They need not be quick screening tests, but could be fairly cumbersome and time consuming, if necessary. The researcher has a generation's time before the animals will be bred, and a test that takes a week or more would be as appropriate as one that takes 5 minutes, although it might indeed be more expensive. Further, the tests need not be especially sensitive. If there is genetic variability in the trait at all, there will be considerable difference among individuals of the original breeding stock, so it will be possible to choose the initial breeders by a rough test. From then on, the lines should diverge, so there should be no difficulty in selecting the desired breeders. For example, the sleep time test used by McClearn and Kakihana (1973) is a relatively crude and imprecise measure of response to ethanol, but it was entirely adequate for the task, because the lines diverged much farther even than the considerable error in the method. Presumably, any useful test will involve some such wide separation of the lines, so a crude test is good enough.

---

*The author's work is supported by grant AA-01066 and Research Scientist Award DA-00048 from the U.S. Public Health Service.

However, we must not be vague as to what we are testing for. The breeding method will allow us considerable precision. We can select for a certain trait (e.g., susceptibility to acute intoxication) while simultaneously ruling out certain uninteresting mechanisms. We could decide whether we want the sleep time to depend on the ethanol elimination rate or on the sensitivity of the brain. In this sense we could use the breeding program to build the control right into the experimental animals. Animals in such programs usually are bred for a certain trait, and the first experiments done with them are to determine the specificity of the trait (other drugs, other behaviors) and to see whether changes in alcohol metabolism rate account for the selected characteristics. With planning, these matters need not be left to chance. It might also be important to avoid getting the same phenotype by two or more different mechanisms. If the mechanisms are known, the researcher could keep one of them constant while varying the other. For example, if we are breeding for an increased rate of ethanol metabolism, we may want to have lines with increased activity of liver alcohol dehydrogenase or hepatic microsomal ethanol-oxidizing system (MEOS), but not both. One further caveat is that we should avoid using any tests that involve learning, unless of course it is learning itself that we are selecting for. Otherwise we will get in the bind just mentioned; that is, the desired phenotype will arise by mixed mechanisms, partly physiological and partly by changed ability to learn the task.

Double testing would be useful for more reliable yield. Whatever test is used, it would be a good idea to run it a second time on the most promising animals, to be sure that there are consistent high and low scorers in the population. In addition, if multiple criteria are to be used, one could first screen for a general effect, then rule out the undesired mechanisms by running the best animals through a second test for specificity.

This chapter concentrates on the main themes of current alcohol research, such as metabolism, acute intoxication, tolerance, and physical dependence. There are established inbred strains that differ with respect to some of these properties, but they are much less valuable than the selectively bred lines. It is an enormous advantage to have lines enriched for the desired trait alone, using outbreeding to keep the other genes scrambled, so that correlations can be sought without having to worry about fortuitous association of other characteristics with the selected one.

Metabolism of Ethanol

Of the many possibilities that come to mind, the most promising is to breed for mice with divergent activities of liver alcohol dehydrogenase. This could be done by measuring the rate of elimination of ethanol from the blood after a test dose. The elimination rate is generally found to be linear with time (zero order) and can easily be determined by taking samples at times when absorption is complete, 90 minutes or more after an intraperitoneal dose. Measuring blood levels of ethanol at 90, 120, and 180 minutes, for example, after a dose of 2 g/kg intraperitoneally should give a good estimate of the elimination rate. One might then want to do a second test on the animals with the highest and lowest rates, to be sure that it was alcohol dehydrogenase activity that determined the rate. This could be done by administering another ethanol dose, this time along with a large dose of pyrazole (4 mmole/kg, i.p.) to inhibit the alcohol dehydrogenase. The elimination rate in pyrazole-treated animals would be the sum of MEOS, catalase, and excretion of the unchanged drug. This should be kept the same from one generation to the next. (Animals with high elimination rates that could not be put down by pyrazole would be very interesting but would not be selected for the alcohol dehydrogenase breeding program.)

Could such selected lines be developed? They probably could, because we know there is considerable variability in ethanol elimination rates within most populations, and presumably some of the variation is genetic. However, it seems strange that among the many strains of rats and mice that have been compared for various alcohol-related characteristics, none seems to vary much from the others with respect to rate of ethanol metabolism. Breeding for sleep time or for ethanol preference should occasionally provide lines that differ in ethanol metabolic rate, if no special precautions were taken to rule this out. But it has not happened yet. There is the interesting possibility that animals need their alcohol dehydrogenase for purposes of which we are ignorant, and it cannot be bred out. In any case, we will have to take into account the possible existence of several isozymes, products of three distinct gene loci in humans. (Smith et al. 1971). The same may be true in other species, although rats and mice show only one band on starch gel electrophoresis (vonWartburg 1971). Once the lines were established, the pattern could be worked out by separating the isozymes with starch gel electrophoresis.

For the breeding itself, one could not easily distinguish between the isozymes, nor would it be necessary. Most of the questions to be asked of these animals apply to any form of alcohol dehydrogenase. They are questions about the role of ethanol metabolism in the acute and chronic actions of ethanol, and they are of two types. First, one might want to know whether acetaldehyde or its derivatives, such as the tetrahydroisoquinolines, may mediate some effects of ethanol. This could be examined with any lines that differ in the rate of ethanol elimination, regardless of the mechanism, because the slowly eliminating lines will have very low or nonexistent circulating acetaldehyde. It will be destroyed by its own degradative enzymes as fast as it is formed. Second, one may want to know whether changes in the liver redox balance are involved. This specifically requires lines that differ in alcohol dehydrogenase activity, because it is the dehydrogenase that consumes the liver's supply of NAD and thus shifts the balance of reduced and oxidized substrates all over the body. A line that was deficient in alcohol dehydrogenase could be used to answer both questions.

We can use these animals to explore the role of alcohol dehydrogenase and/or MEOS in the production of fatty liver or other hepatic pathology. In this type of study, whenever any response to ethanol is compared between lines with different rates of alcohol elimination, it is essential to equalize the blood alcohol levels by adjusting the doses.

The lines that differ in dehydrogenase activity will have very different blood ethanol concentrations after a standard dose of ethanol, and the slow-metabolizing line may have much greater hepatic damage simply for that reason. It would be a serious misuse of these animals to fail to control for this. It will be necessary to determine the doses that give rise to the same alcohol exposure (blood concentration × time) for each line. When this is done, there should be two groups of animals receiving the same exposure to ethanol but very different exposure to acetaldehyde and very different shifts in NAD/NADH balance. This would facilitate sorting out the many variables participating in ethanol's actions on hepatic lipid metabolism.

Many of the peripheral actions of ethanol and some central effects seem to be due to stress, a response that may be mediated by acetaldehyde. So it would be interesting to see whether the blood glucocorticoid response to ethanol still occurs in the slow-metabolizing animals. Also, acetaldehyde apparently has direct effects on the adrenal medulla and on sympathetic nerve endings, releasing catecholamines. What would the intoxicated animal look like if these sympathomimetic effects were absent? Some investigators will want to study alcohol preference in these animals, because the slow metabolizers will presumably lack the ability to form tetrahydroisoquinolines, which are currently being investigated as possible mediators of excessive voluntary alcohol intake (Myers and Melchior 1977). We are getting very interesting mixed messages about acetaldehyde and its role in alcohol preference. On the one hand, acetaldehyde seems to account for the aversive properties of ethanol in genetic studies of alcohol consumption by rats and mice. On the other hand, acetaldehyde is the source of tetrahydroisoquinolines, whose striking positive effect on voluntary ethanol intake has recently been demonstrated by Myers and Melchior (1977).

Many of these same questions could be answered by using t-butanol, an alcohol that produces intoxication and physical dependence much like ethanol but is not converted to acetaldehyde. Experiments with the proposed metabolism lines would complement the t-butanol work but would be more directly relevant to alcoholism because ethanol itself would be used.

Animals that metabolize ethanol very slowly would have advantages and disadvantages for the study of physical dependence. A state of continuous intoxication, which seems to be a sine qua non for producing physical dependence, is difficult to attain in rodents. The ability to maintain stable blood ethanol concentrations with daily injections would be welcome. We would have an easy time evoking physical dependence in such lines, but we would have a hard time showing that we had done it. The problem is that there would be no way to remove the block on metabolism in order to withdraw the animals. The slow elimination would taper off the blood levels and mask the withdrawal reaction. It would be like trying to show physical dependence on a long-acting barbiturate or a benzodiazepine. It can be done, but the withdrawal reaction is unimpressive. Boisse and Okamoto (1978) measured the intensity of barbiturate withdrawal reactions, comparing fast and slow elimination. They found, as might be predicted, that fast elimination gives a stronger withdrawal reaction. We could make such a comparison with lines that differ in rate of ethanol metabolism, confirming Okamoto presumably, and perhaps go a bit further to examine quantitatively the relation between withdrawal scores and blood ethanol levels, but there seems little more to do on this topic.

Acute Intoxication

Behavioral Signs

There are several straightforward tests of ataxia, motor activity, etc., that could easily be used to assess the degree of acute intoxication in animals and thus select particularly sensitive or resistant indivduals for breeding. Some tests that give clear numerical data and are reliable will be indicated. As mentioned above, it does not seem appropriate to use tests that involve learning, because genes may affect the learning of a task as well as the performance of it. For example, the rotarod test (Dunham and Miya 1957) would not be useful because mice learn to walk the rotating rod in an intoxicated condition, which complicates not only the initial test but also any future study of tolerance to it. The necessity of training the animals to stable performance criteria is a big disadvantage of the otherwise valuable moving belt test (Gibbins et al. 1968) and some others. As tests of ataxia, the simplest is recommended, either the tilting plane test (Arvola et al. 1958) or the dowel test (Gallaher and Goldstein 1978). The former is simply a matter of placing a rat or mouse on a hinged board and raising one end until the animal slides off. The angle attained by each animal, expressed as a percentage of its predrug angle, is that animal's score. The dowel test is a stationary rotarod. A mouse is placed on a horizontal dowel, 18 inches above a soft surface. A sober mouse stays on indefinitely, walking around, grooming itself, and occasionally inspecting the under side of the bar. But a drunk mouse falls off, promptly and unmistakably. As it sobers up, it can be tested occasionally, and there is an easily definable point at which it regains the ability to balance on the dowel for 30 seconds. A tail blood sample is taken at that point for ethanol assay, and this threshold blood ethanol concentration is the score for that animal, the variable for which the breeding would be carried out. Practically, a dose of 2 g/kg i.p. will cause most Swiss-Webster mice to lose their dowel prowess for about half an hour, at which time their blood alcohol level is about 2 mg/ml. It is important to use a high enough dose to allow time for the blood and brain to equilibrate before the threshold is reached; otherwise it is no use looking at blood levels. Only the brain concentration would be meaningful, and that is inaccessible. Too high a dose, on the other hand, would also be a poor choice, because acute tolerance might develop during the time of action of a single dose, as discussed below.

Analytical methods are not included in this chapter, but it should be pointed out that blood ethanol is the easiest of assays. Enzymatic methods for ethanol determination are simple, require minimal training of technicians, and can be done efficiently on dozens of samples at a time. An enzymatic method would be more efficient than gas chromatography for this purpose.

Another measure of acute intoxication is gross motor activity. Lester's group (Riley et al. 1976) has used the ethanol-induced decrease in motor activity, measured with a stabilimeter platform, to separate two lines of rats, designated most affected (MA) and least affected (LA). The differential reactivity was found to extend to sleep time as well (Riley et al. 1977). This is not necessarily the outcome of any subsequent breeding program—the test chosen for reactivity may not give the same line differences as other tests that seem very similar. For example, the long-sleep (LS) and short-sleep (SS) mouse lines that differ in sleep time do not differ in the rotarod test (Sanders 1978). It is certainly difficult to predict which may be the most meaningful test. It might be best to use more than one, if a general differential sensitivity is desired. One could select for traits that vary in parallel during the breeding program. The best way to go might be to insist on pharmacological specificity, rather than on behavioral specificity. That is, the lines should diverge with respect to ethanol effects, not the effects of other drugs. One could choose animals whose ethanol scores were either the highest or lowest *and* whose pentobarbital scores were within some predetermined range. In this way, we aim for genetic differences close to the primary action of the drug. Barbiturates and ethanol produce almost identical behavioral patterns. Presumably, they affect different sets of neurons whose ultimate projection is onto the same "final common path." This assumption is itself a deduction from reports that strains with different sensitivities to ethanol do not necessarily differ in response to barbiturates (Erwin et al. 1976; Randall and Lester 1974; Sanders et al. 1978; Siemens and Chan 1976). This has been one of the most important results of the LS/SS breeding program. There are possible trivial reasons for this differential sensitivity (effects on transport, for example), but they are unlikely. Probably the drugs have separate sites of action, under separate genetic control, and the breeding program has hit close to the real target rather than at some more distant site along the pathway.

Physiological Measures

Physiological tests have some important advantages over behavioral ones. They are easier to use for studies of tolerance because there can be no learning (although there may be some other kinds of conditioning or adaptation). The variables most clearly affected by ethanol are body temperature and respiration. Temperature is the easier one to measure and has been used for studies of tolerance, particularly by Ritzmann and Tabakoff (1976). Body temperature can be determined repeatedly in the same animal, to show a time course or establish the maximum effect. It is much eaier to measure than to interpret, because the body temperature in mammals is under elaborate central control, involving most, if not all, of the known neurotransmitters. The hypothermic effect does not easily indicate what neurochemical system has been disrupted.

Respiration is more complex to measure, because we have to decide what aspect to look at. Probably the most senstive and specific is the response to CO_2, because this is known to be depressed even when the respiratory rate or minute volume has compensated. This should be simple enough to measure, but there is not much literature on the magnitude of the ethanol effect or its normal variability. It does seem worth investigating as a new test for ethanol action. However, we must expect that the essential respiratory control mechanisms will be protected by an elaborate and redundant neuronal system that will not be easy to analyze when we attempt to find the mechanism of a genetic effect. If we did have lines that differed in sensitivity of the respiratory center to ethanol, it is likely that they would be generally useful for physiologists and pharmacologists outside the alcohol field. Selected lines with spinoff value should be encouraged.

Another set of lines might be developed according to their degree of general CNS excitability, rather than any response to ethanol. This seems appropriate because we are dealing with a sedative drug. For selection, one could pick any of several measures of excitability known to be affected by ethanol. Those indexes commonly used to assess withdrawal hyperexcitability would be suitable. They include startle threshold (Gibbins et al. 1971) or seizure threshold with pentylenetetrazol or electroshock (McQuarrie and Fingl 1958) or flurothyl (Greer et al. 1976). One could also use the reliable convulsions on handling that can occur in mice in response to very low doses of almost any convulsant drug (Goldstein 1973*a*). People who study anticonvulsant medications or the causes of epilepsy might be interested in these lines, especially because there is a known genetic component in some of the epilepsies.

Finally, it would be worthwhile to repeat the selection of lines differing in sleep time, because it is very clearly feasible in mice. Done a second time, the constriction in the long-sleep line could be avoided and the new lines would be more useful for study of biochemical or behavioral correlates of sleep time.

Tolerance

It would be feasible to breed for the differential development of tolerance to ethanol. For this, we have to decide whether we want acute or chronic tolerance and how to be sure that we have only functional tolerance. We surely need functional tolerance, which is a changed sensitivity of the brain to ethanol. The alternative is dispositional or metabolic tolerance, which could be dealt with by methods analogous to those described above for studies of elimination rate. There are two ways to be sure that the observed tolerance is functional. One way is to determine a threshold blood alcohol level for some behavioral endpoint. One would measure blood levels of ethanol at the time the effects were ascertained. This must be done long enough after ethanol administration so that blood levels accurately represent brain concentrations—about 30 minutes after i.p. administration, for example. At earlier times, the brain has higher levels than venous blood, because the brain equilibrates rapidly with arterial blood, but there is still an arteriovenous difference in the periphery. The other way is to make the behavioral measurement so soon after administration that the metabolic rate cannot affect the brain concentration. This is the approach used by LeBlanc et al. (1969) in studies of chronic tolerance. They tested rats on the moving belt apparatus 23 minutes after administration of ethanol. Here the tolerance is measured in terms of the behavioral change at a presumably uniform brain level.

The decision whether to breed for acute or chronic tolerance will depend on what price the researcher is willing to pay. Chronic tolerance is probably of greater general interest, because it is a known component of alcoholism and is familiar to researchers. It would be feasible to screen for chronic tolerance in either rats or mice, using somewhat different dosing regimens. For rats, one could use LeBlanc's daily intubation regimen, with increasing doses over a 2-week period (LeBlanc et al. 1969). This has been shown to produce tolerance on the moving belt task and requires attending to the animals only once a day. For mice, Ritzmann and Tabakoff (1976) have been successful with a liquid diet technique. However, they used a commercial diet preparation (Slender), which is not ideal because its composition may change in the future or it may not even be available. A defined diet would be preferable, but the exact conditions for use of a new diet would have to be worked out. Anyone who has done this knows it takes time. Different inbred strains need slightly different concentrations of ethanol in the diet for optimal intake. A little too much ethanol reduces consumption (both the intake of diet and of absolute ethanol) and causes loss of body weight, while slightly too little ethanol allows the mice to drink less than they can metabolize so the blood levels remain undetectable. The margin is narrow. It might be difficult to change the conditions progressively as the degree of tolerance diverges between lines. Such procedures may be feasible, but they would not be easy.

In any of the models for chronic tolerance, the end result could be assessed with any of the tests suggested above for measurement of acute intoxication. If desired, the researcher could use more than one such test to select for a general type of tolerance.

Acute, rapidly developing tolerance would probably be a better trait to select for, despite its less certain relevance. It is easy, accurate, and susceptible to little or no learning artifact. Acute tolerance can be measured with repeated trials on the dowel task (Gallaher and Goldstein 1978). Each time the mouse reaches threshold (regains ability to balance on the dowel), another small dose of ethanol is given, 1 g/kg or half the initial dose. This reinstates the intoxication; the mouse loses its ability to balance. Blood samples are taken when balance is regained after each of five doses. The threshold rises from about 2 mg/ml to 3 mg/ml over a period of about 6 hours (Swiss-Webster mice). To save time and to prevent practice effects (which are negligible with Swiss-Webster mice but might occur in other strains), one can give the five injections on a predetermined schedule and measure the threshold only after the first and fifth injections. The datum for each mouse will be the rise in threshold, if any. One could test for acute tolerance with other tasks or with the loss of righting reflex, as Littleton has done (Grieve et al. 1979). Littleton's very interesting method is as follows: Mice are exposed to a high concentration of ethanol vapor in an apparatus where they can be handled. They are removed for an alcohol assay when they lose their righting reflex or fall off a rotarod. Tail blood might not represent brain levels at such early times, so ethanol is assayed in the expired air, using a simple mouse holder and an empirical calibration curve. The alcohol exposure and assay at threshold are repeated several times. Littleton finds that C57BL mice develop tolerance much faster than DBA mice do. Their initial sensitivity, which is measured with Littleton's method much better (earlier) than with that of Gallaher and Goldstein (1978), appears to be the same in both strains.

It is already evident that different strains of mice develop acute tolerance at different rates. To analyze a line difference in rate of tolerance production is too cumbersome for a screening procedure—it is a major investigation in itself. The best way around this problem is to simply select mice on the basis of their tolerance at a given time, without concern for how fast they got there or whether they might go further.

The lines that develop functional tolerance to different extents would be useful for studying the biochemical correlates of tolerance, as well as the relations between acute and chronic tolerance and between tolerance and dependence. These animals would be used for neurochemical studies, including turnover of neurotransmitters and effects of 6-hydroxydopamine, which blocked tolerance in Tabakoff's experiments (Tabakoff and Ritzmann 1977). For biophysical pharmacologists, the physical state (fluidity) of the cell membranes will be of interest, as well as the change in membrane fluidity with ethanol in vitro, and any associated differences in the chemical composition of the membrane lipids.

Physical Dependence

Genetic evidence indicates that researchers can easily breed for differences in severity of withdrawal reactions, but that does not necessarily mean that they have differences in susceptibility to physical dependence. In the author's laboratory we have bred Swiss-Webster mice for intensity of convulsions-on-handling scores in alcohol withdrawal. Only one generation sufficed to show (and another to confirm) that progeny of high-scoring mice had higher seizure scores than progeny of low-scoring mice, after a standard cycle of ethanol intoxication (Goldstein 1973*b*). Goldstein and Kakihana (1974) showed a similar differential sensitivity to withdrawal seizures in C57BL versus DBA mice. The C57BL mice showed almost no such convulsions, but we know that they do become dependent on ethanol. Others have shown a variety of withdrawal signs, including spontaneous convulsions in C57BL mice after chronic treatment with ethanol. Littleton, who scores piloerection, tail lift, and convulsions on handling, observed a strong withdrawal reaction in DBA mice but almost none in C57BL (Grieve et al. 1979). The difference between the dependent state and the withdrawal reaction becomes clear here. Two strains may be equally dependent, in some unknown biochemical sense, but may have different severity of outward signs of the dependent state. Therefore, while it would be feasible to breed for severity of withdrawal signs, using any of several indexes of CNS hyperexcitability or a combination of them, it would not be correct to consider that there was differential physical dependence. This would greatly limit the usefulness of these mice, although it would indeed be interesting to explore neurochemical and biophysical correlates of the seizure susceptibility.

If it were to be done, the way to begin would be to breed for susceptibility to the earliest signs of physical dependence, which can be elicited in some mice by a single dose of ethanol (Goldstein 1972; McQuarrie and Fingl 1958). One could use withdrawal seizure susceptibility (e.g., with pentylenetetrazol, mild electroshock, flurothyl, or simply handling). The reason the "acute dependence" seems to be a reasonable test is that accumulation of physical dependence seems to begin with the initiation of exposure to ethanol and to continue progressively with chronic exposure (Goldstein 1972). One could easily select increased, but not decreased, susceptibility to convulsions. To find those animals that are less prone to withdrawal seizures, we would have to use a longer period of ethanol administration, enough so that almost all the mice would convulse, then pick out those with the lowest scores. For chronic administration of ethanol one could use the Majchrowicz method (Majchrowicz and Hunt 1976) for rats, i.e., give ethanol a few times a day, adjusting each animal's dose according to its behavioral condition. This allows nicely for variations in response that might arise with development of tolerance. I do not know a suitable system for mice; it may not be wise to use the inhalation model with pyrazole (Goldstein 1972). Inhalation without using pyrazole to stabilize the blood ethanol concentrations is tricky and variable. Liquid diets, such as those used by Ritzmann and Tabakoff (1976), should work, once the conditions are worked out, but these diets are difficult to regulate.

It seems of dubious worth to embark on a program for selective breeding related to physical dependence at this time. With unlimited resources it might be done some day, but there seem more promising models at the moment.

Conclusions

It is reassuring to know that the breeding of valuable research animals is in the hands of experienced geneticists. The procedures of selective breeding phenotypes in each generation should be carried out with very clear ideas as to exactly what is being selected. The possibility of "breeding in the controls," so that the animals end up with the right traits for the right reasons, should be considered, balancing the costs of repeated testing against the advantages of phenotypic specificity.

References

Arvola, A.; Sammalisto, L.; and Wallgren, H. A test for level of alcohol intoxication in the rat. *Q J Stud Alcohol*, 19:563-572, 1958.

Boisse, N.R., and Okamoto, M. Physical dependence to barbital compared to pentobarbital. IV. Influence of elimination kinetics. *J Pharmacol Exp Ther*, 204:526-540, 1978.

Dunham, N.W., and Miya, T.S. A note on a simple apparatus for detecting neurological deficit in rats and mice. *J Am Pharm Assoc*, 46:208-209, 1957.

Erwin, V.G.; Heston, W.D.W.; and McClearn, G.E. Effects of hypnotics on mice genetically selected for sensitivity to ethanol. *Pharmacol Biochem Behav*, 4:679-683, 1976.

Gallaher, E.J., and Goldstein, D.B. The onset of tolerance to ataxic effects of ethanol in the mouse. (Abstr.) *Alcoholism: Clin Exp Res*, 2:194, 1978.

Gibbins, R.J.; Kalant, H.; and LeBlanc, A.E. A technique for accurate measurement of moderate degrees of alcohol intoxication in small animals. *J Pharmacol Exp Ther*, 159:236-242, 1968.

Gibbins, R.J.; Kalant, H.; LeBlanc, A.E.; and Clark, J.W. The effects of chronic administration of ethanol on startle thresholds in rats. *Psychopharmacologia*, 19:95-104, 1971.

Goldstein, D.B. Relationship of alcohol dose to intensity of withdrawal signs in mice. *J Pharmacol Exp Ther*, 180:203-215, 1972.

Goldstein, D.B. Convulsions elicited by handling: A sensitive method of measuring CNS excitation in mice treated with reserpine or convulsant drugs. *Psychopharmacologia*, 32:27-32, 1973*a*.

Goldstein, D.B. Inherited differences in intensity of alcohol withdrawal reactions in mice. *Nature*, 245:154-156, 1973*b*.

Goldstein, D.B., and Kakihana, R. Alcohol withdrawal reactions and reserpine effects in inbred strains of mice. *Life Sci*, 15:415-425, 1974.

Greer, C.A.; Alpern, H.P.; and Collins, A.C. Increased CNS sensitivity to flurothyl as a measure of physical dependence in mice following morphine, phenobarbital and ethanol treatment. *Life Sci*, 18:1375-1382, 1976.

Grieve, S.J.; Griffiths, P.J.; and Littleton, J.M. Genetic influences on the rate of development of ethanol tolerance and the ethanol physical withdrawal syndrome in mice. *Drug Alcohol Depend*, 4:77-86, 1979.

LeBlanc, A.E.; Kalant, H.; Gibbins, R.J.; and Berman, N.D. Acquisition and loss of tolerance to ethanol by the rat. *J Pharmacol Exp Ther*, 168:244-250, 1969.

Majchrowicz, E., and Hunt, W.A. Temporal relationship of the induction of tolerance and physical dependence after continuous intoxication with maximum tolerable doses of ethanol in rats. *Psychopharmacology*, 50:107-112, 1976.

McClearn, G.E., and Kakihana, R. Selective breeding for ethanol sensitivity in mice. (Abstr.) *Behav Genet*, 3:409-410, 1973.

McQuarrie, D.G., and Fingl, E. Effects of single doses and chronic administration of ethanol on experimental seizures in mice. *J Pharmacol Exp Ther*, 124:264-271, 1958.

Myers, R.D., and Melchior, C.L. Alcohol drinking: Abnormal intake caused by tetrahydropapaveroline in brain. *Science*, 196:554-555, 1977.

Randall, C.L., and Lester, D. Differential effects of ethanol and pentobarbital on sleep time in C57BL and BALB mice. *J Pharmacol Exp Ther*, 188:27-33, 1974.

Riley, E.P.; Freed, E.X.; and Lester, D. Selective breeding of rats for differences in reactivity to alcohol. An approach to an animal model of alcoholism. I. General procedures. *J Stud Alcohol*, 37:1535-1547, 1976.

Riley, E.P.; Worsham, E.D.; Lester, D.; and Freed, E.X. Selective breeding of rats for differences in reactivity to alcohol. An approach to an animal model of alcoholism. II. Behavioral measures. *J Stud Alcohol*, 38:1705-1717, 1977.

Ritzmann, R.F., and Tabakoff, B. Body temperature in mice: A quantitative measure of alcohol tolerance and physical dependence. *J Pharmacol Exp Ther*, 199:158-170, 1976.

Sanders, B. Sensitivity to low doses of ethanol and pentobarbital in mice selected for sensitivity to hypnotic doses of ethanol. *J Comp Physiol Psychol*, 90:394-398, 1976.

Sanders, B.; Sharpless, S.K.; Collins, A.C.; McClearn, G.E.; and Flanagan, C. Activating and anesthetic effects of general depressants. *Psychopharmacology*, 56:185-189, 1978.

Siemens, A.J., and Chan, A.W.K. Differential effects of pentobarbital and ethanol in mice. *Life Sci*, 19:581-589, 1976.

Smith, M.; Hopkinson, D.A.; and Harris, H. Developmental changes and polymorphism in human alcohol dehydrogenase. *Ann Hum Genet*, 34:251-271, 1971.

Tabakoff, B., and Ritzmann, R.F. The effects of 6-hydroxydopamine on tolerance to and dependence on ethanol. *J Pharmacol Exp Ther*, 203:319-331, 1977.

von Wartburg, J.-P. The metabolism of alcohol in normals and alcoholics: Enzymes. In: Kissin, B., and Begleiter, H., eds. *The Biology of Alcoholism.* Vol. 1. New York: Plenum Press, 1971.

Multivariate Methodology

William Meredith

This chapter presents and describes briefly two related families of multivariate analysis. Some definitions are in order. By multivariate analysis is meant the statistical analysis of systems of interrelated variables. Such analysis may be descriptive or inferential (Anderson 1958; Finn 1974; Morrison 1976). Systems of interrelated variables occur frequently in scientific work for at least three different reasons.

First, it is a commonplace that most experimental manipulations, in biological and behavioral work at least, affect many more potentially observable variables than the one, or few, examined by a particular scientist. Experimenters' choice of dependent and independent variables is limited by their knowledge and imagination, is in considerable measure dictated by fashion, and to a large degree is determined by the availability and precision of observational or measurement procedures. For example, let us suppose the experimental intervention consists of the administration of some drug. The drug would have a number of potentially observable physiological consequences such as an alteration in heart rate and oxygen-carrying capacity of the blood; these two consequences are themselves interrelated. A simple behavioral effect might be a change in respiration rate, and other measurable changes (for example, in cognitive function, reaction time, and mood) could presumably be reliably assessed. Furthermore, the whole system might be modified and mediated by individual differences in tolerance, habituation, and specific metabolic processes.

How can we analyze such a complex of interrelated variables? Obviously it would be a formidable task, and the statistical analysis of such systems is only beginning to develop. But we do have a family of techniques, collectively referred to as path analysis or linear structural relations analysis, that can be employed in such situations provided the experimenter is able to specify the nature of the significant variables, is able to measure them reliably, and, *most important*, has a hypothesis about the causal network of the system (Jöreskog 1973; Kerlinger and Pedhazur 1973; Namboordi et al. 1975; Wright 1967).

The second situation, and perhaps the most important of the three that motivate multivariate analysis, arises from the fact that behavioral scientists usually do not know *exactly* what they want to study; or, at least, they are unable to agree on operational definitions of central variables. Let us consider, for example, the experimenter who wishes to study activity in mice. A quick review of the literature would reveal that there are at least a dozen different devices and/or techniques that have been systematically employed in the study of rodent activity by different investigators. Yet we commonly act as if they were all studying the same thing. Whether or not different techniques are interchangeable is an open question that needs to be answered empirically, rather than by fiat or intuition.

This problem has a long history in psychology with its roots in early studies of intellectual or cognitive functioning in humans. The methodologies psychologists developed to study the complexity of cognitive functioning are known collectively as latent trait analysis or factor analysis. These techniques have been widely applied in the behavioral sciences in an almost unlimited variety of substantive areas (Harman 1967; Jöreskog 1970; Lazarsfeld and Henry 1968; Mulaik 1972).

The third reason that multivariate situations arise in scientific work is that any attempt to study behavior longitudinally or developmentally almost by definition implies multiple variables, at least multiple observations, on the same developing attribute in the same individuals. (We will not discuss the class of solutions to such problems currently available.)

A fourth reason for the occurrence of multivariate systems in scientific work is due to "multiple variates of convenience." By this I mean that investigators often find it easy to record certain observations without having any theoretically compelling reason to do so. For example, in any study of disease processes one would automatically record heights and weights of subjects. In a learning experiment one can easily record latency of response, correctness of responses, sometimes vicarious responses, etc., on each learning trial. There are a variety of extensions of familiar methods of statistical analysis (e.g., analysis of variance) that can be straightforwardly applied in such situations (Finn 1974; McNemar 1969; Scheffe 1959).

The two kinds of situations to be characterized here briefly—linear structural relations analysis and latent structure analysis—are both grounded in multiple regression analysis, in correlational theory, and in the theory of linear composite measures. Let us begin with a recapitulation of basic multiple regression theory and correlation theory. (I presume we are all familiar with the principles of univariate sample regression.)

Let the dependent variable be denoted by y, the predictor variable by x; we wish to express the relation of y to x by $y = Bx + \Theta$ in such a way that the average of $(y-(\beta x + \theta))^2$ is minimized; that is, we are fitting a line by least squares (L.S.). It is not widely appreciated how general the foregoing formulation is. The variables x and y may be of almost any sort—continuous, discrete, even qualitative with suitable "dummy" coding. The predictor variable may be fixed (i.e., controlled by the experimenter) or random (i.e., naturally varying in some population of organisms). (Fitting a dosage-response curve using logits or probits is often essentially an exercise in linear regression.)

The correlation coefficient between x and y, ρ_{xy} , is the ratio of the covariance to the product of the standard deviations, that is, $\rho_{xy} = \sigma_{xy}/\sigma_x\sigma_y$. Correlation and regression are intimately interconnected by the well-known results, $\beta = \rho_{xy}\sigma_y/\sigma_x$, $\theta = \mu_y - (\rho_{xy}\sigma_y/\sigma_x)\rho_x$, and $\sigma^2_{y\cdot x} = \epsilon(y - x\beta - \theta)^2 = \sigma^2_y(1 - \rho^2_{xy})$. In the foregoing, σ^2 denotes a variance and σ a covariance according to the subscript, μ denotes a mean, and ϵ denotes the process of averaging or computing an expected value.

Furthermore, if we write $\widetilde{y} = \beta x + \theta$, then $\rho_{\widetilde{y}y} = \rho_{yx}, \sigma^2_y = \rho^2_{yx}\sigma^2_{\widetilde{y}}$, $\rho_{xy} = 1, \sigma^2_y = \sigma^2_e + \sigma^2_y$, where $e = y - y . \epsilon(y - y)^2 = \sigma^2_e$, and $\rho_{ex} = 0$. If we express both x and y in standard units (standard scores), say z_{y2} and z_x, then the L.S. regression becomes $z_{\widetilde{y}} = \rho_{xy}z_x$ to minimize $\epsilon(z_y - z_{\widetilde{y}})$.

Now suppose we have not one predictor variable, but many (say, $x_1, x_2, \ldots, x_p$). We proceed by constructing a weighted linear composite of these x's (say, $\widetilde{y} = \Sigma\beta_i x_i + \theta$). Note: in simple regression the data points come as pairs, each pair being a joint observation on y and x "simultaneously." In multiple regression the data points come as $(p + 1)$ -tuples, each $(p + 1)$ -tuple being composed of $p + 1$ joint observations. As in the simple regression case, we can choose the weights $\beta_1, \beta_2, \ldots, \beta_p$ and θ so that $\epsilon(y - \widetilde{y})^2 = \epsilon(y - (\Sigma\beta_i x_i + \theta))^2$ is minimized. The formulas for the multiple regression weights are not simple and need not concern us extensively here. An exception can be made for the case in which the predictor variables are statistically uncorrelated or designed to be orthogonal in an experiment. In these cases each regression weight can be obtained by $\beta_i = \rho_{x_i y}\sigma_y/\sigma_x$. In either event the previous results go through—for example, $\epsilon(y - \tilde{y})^2 = \sigma_y^2(1 - \rho_{\tilde{y}y}^2)$. The correlation $\rho_{\tilde{y}y}$ is called the multiple correlation, and the signs of the weights are chosen so that the multiple correlation is always positive.

We remark that all analysis of variance designs can be handled as special cases of multiple regression by introducing "dummy coded" variables for the predictor variables which in combination designate an individual subject's membership in a treatment group (Finn 1974; Kerlinger and Pedhazur 1973). Using orthogonal contrasts in such "dummy coding" so that the predictors are orthogonal, we find that each regression coefficient can be interpreted as an "effect" in traditional analysis of variance terms, and the F test for a selected subset, S, of K orthogonal effects becomes simply

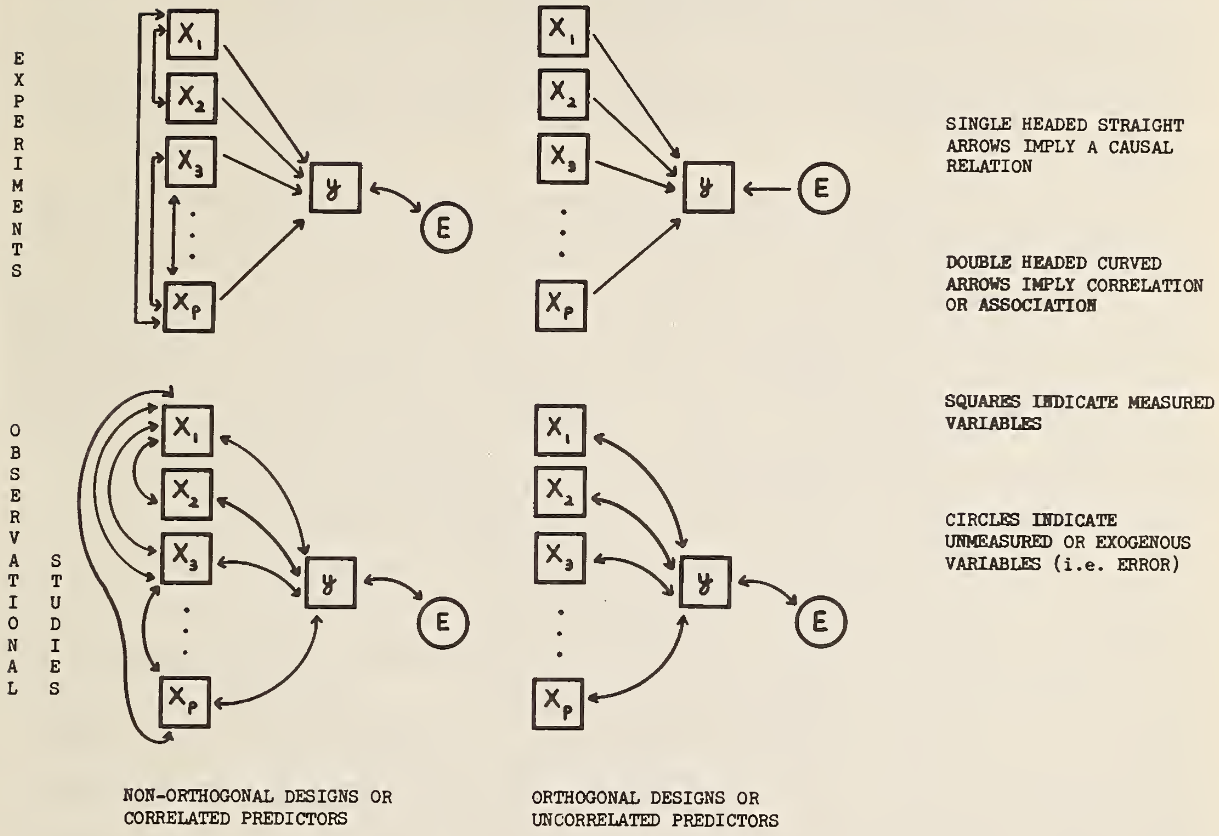
$$F = \left\{ \sum_{S} \beta_i^2 / K / o_e^2 \right\}$$

making appropriate adjustments for sample size and sample parameters. The notation $\tilde{\beta}$ denotes a sample L.S. estimate, and o_e^2 denotes the sample variance about regression. Analysis of covariance can be handled by mixing dummy coded predictors and measured control variables.

Regression analysis can be formulated as an exercise in path analysis (Kerlinger and Pedhazur 1973; Namboordi et al. 1975; Wright 1967), as presented in figure 1 for various cases. Whether or not a path is to be interpreted as indicating a causal effect or an association depends on whether the experimenter directly manipulated the predictor variable or whether variability in the predictor is due to random sampling from a naturally varying population. The strength of a causal effect or an association can be indexed by the regression coefficient; usually we use standardized regression coefficients $\alpha_i = \beta_i \sigma_{x_i} / \beta_y$. Standardized regression coefficients are often called path coefficients (Wright 1967). Note that the standardized weight is simply $\rho_{x_i y}$ if the predictors are uncorrelated; otherwise it is a partial covariance for standardized scores. A partial correlation coefficient is the correlation between residuals from predicting y and x_i after both y and x_i have been predicted linearly on the basis of $x_1, x_2 \ldots, x_i - 1, x_i + 1, \ldots, x_p$ (i.e., all variables except y and x_i). A partial covariance is the numerator of a partial correlation.

In path analytic models the residuals are often introduced as exogenous variables (indicated by E 's in figure 1), often composed of what we usually relegate to error in discussions of analysis of variance (Jöreskog 1973).

Figure 1. Multiple Regression as a Path Model


EXPERIMENTS
OBSERVATIONAL STUDIES
X1
X2
X3
Xp
y
E
SINGLE HEADED STRAIGHT ARROWS IMPLY A CAUSAL RELATION
DOUBLE HEADED CURVED ARROWS IMPLY CORRELATION OR ASSOCIATION
SQUARES INDICATE MEASURED VARIABLES
CIRCLES INDICATE UNMEASURED OR EXOGENOUS VARIABLES (i.e. ERROR)
NON-ORTHOGONAL DESIGNS OR CORRELATED PREDICTORS
ORTHOGONAL DESIGNS OR UNCORRELATED PREDICTORS

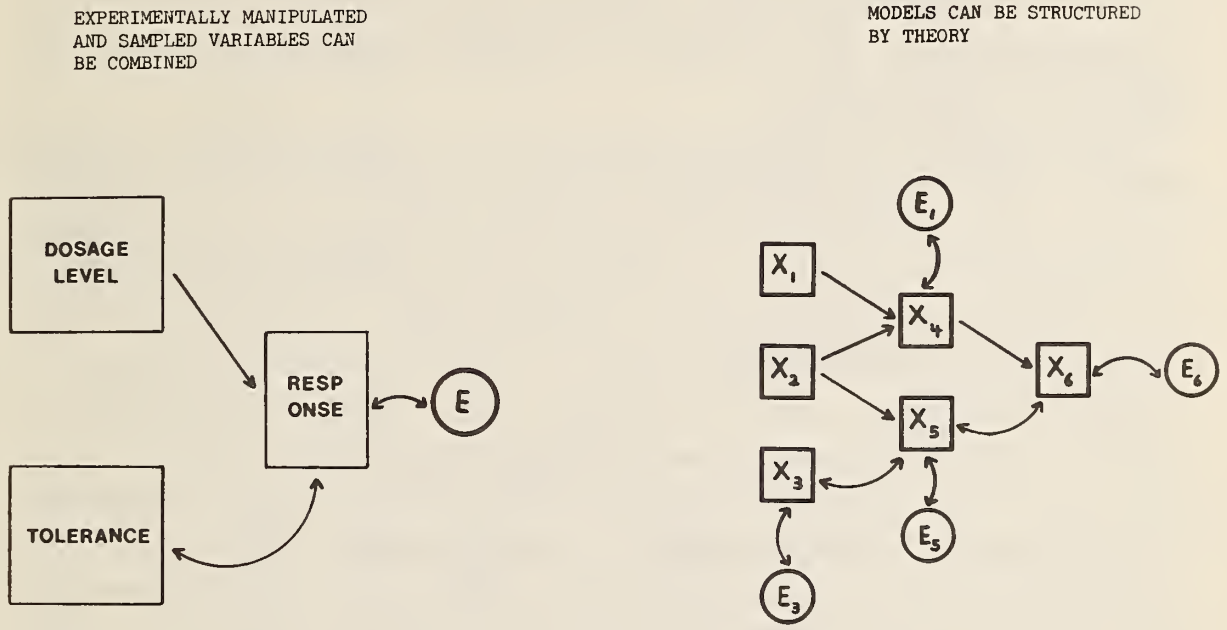
Methods exist for testing the hypothesis that any regression weight (path coefficient) or subset of weights is zero. These reduce to comparing estimated variance about regression, that is o_e^2, calculated with the subset of variables with zero paths deleted, to the corresponding variance for the full model. Preset values for path coefficients determined by hypotheses can be tested in much the same way. Note that because $o_e^2 = o_y^2(1 - \rho_{yy}^2\sim)$, , this always can be viewed as a comparison of the two multiple correlations (Finn 1974; Morrison 1976). Some alternate tests employ the regression weight directly via its sampling distribution.

Now we might think of having multiple, say q , dependent measures $y_1, y_2, \ldots, y_q$. This, it turns out, poses no new problem for the investigator until we get to the level of testing hypotheses about what are now vectors or matrices of regression weights. To calculate the regression weights or path coefficients one simply deals with each dependent variable, y_i , singly in turn. The testing of hypotheses becomes somewhat problematical because potentially q of them can be carried out, one for each dependent variable, and the probability of at least one type I error becomes high. It is preferable to use a multivariate test statistic in which whole variance-covariance matrices, whose typical element would be the covariance $o_{e_ie_j}$, are compared. Such multivariate tests are reasonably well known by now and give control of the probability of type I error while being more powerful or sensitive than a series of univariate hypotheses tests due to the compounding of correlated information (Finn 1974; Morrison 1976).

In many situations, except possibly for direct experimental manipulations, the distinction between dependent and independent variables becomes difficult to maintain even though there is only one end effect variable of primary interest. Figure 2 suggests schematically how models could be constructed with such a distinction obscured. On the right of the figure, x_1 and x_2 could be two experimental variables (say, dosage level and genotype) in a comparison or two strains; x_3 could be a varying attribute, such as body weight, expressed as a deviation score within a strain and hence uncorrelated with x_1 by design (randomization of treatment) and x_2 by construction. The variables x_4 and x_5 could measure two metabolic processes or rates, both affected by genotype but only one, x_4 , affected by dosage. Finally, x_6 denotes some behavioral or physiological end effect affected by both x_4 and x_5 . The E's denote errors of measurement and, in the case of E_6 , probably exogenous variables as well. Because the effects of x_1, x_2, x_3 on x_6 are all mediated through x_4 and x_5 , the multiple regression coefficients for x_1, x_2, and x_3 should all be zero. Furthermore, the simple correlation between x_1 and x_6 , what we might call the unadjusted path coefficient, must be the product of the separate path coefficients from x_1 to x_4 and x_4 to x_6 , or simply $\rho_{x_1x_6} = \rho_{x_1x_4}\rho_{x_4x_6}$. Similar statements can be made for the paths from x_2 to x_6 and x_3 to x_6. Such models may be tested and evaluated by direct investigation of specific predictions such as the foregoing or can be fitted and evaluated in one operation by using computer programs such as Jöreskog's LISREL (Jöreskog 1973) or similar programs that either maximize the likelihood of the data under the hypothesized model or minimize the entire sum of squared residuals.

Now for a system of p variates there are 2^p simple paths possibly connecting the variables. This means path analysis is not very useful in the exploratory phases of research. If no constraints are imposed on the paths, it reduces to ordinary multiple regression analysis, which is often not particularly informative about what is going on. Stepwise multiple regression is frequently employed to sort out exploratory research, but this has real hazards unless the variables can be ordered a priori—for example, temporally or in theoretical priority. Even here it has interpretive hazards. To return to figure 2, we observe that x_2 might be the primary and most important

Figure 2. **Construction of Path Analytic Models for Experimentally Manipulated and Sampled Variables**


EXPERIMENTALLY MANIPULATED AND SAMPLED VARIABLES CAN BE COMBINED
MODELS CAN BE STRUCTURED BY THEORY
DOSAGE LEVEL
RESP ONSE
E
TOLERANCE
E1
X1
X4
X2
X6
E6
X5
X3
E5
E3

determinant of x_6. If it were selected first in a stepwise procedure, the importance and significance of x_4 and x_5 would be diminished and either x_1 or x_3 might be the next selection. In other words, stepwise analysis is not designed to reveal the structure exhibited in figure 2, although it might.

The most powerful exploratory techniques yet devised seem to be factor analytic in orientation. There is an enormous literature on factor analysis and many different techniques have been devised for latent trait theory, factor analysis, component analysis, and latent structure analysis. Only the main details of factor and component analysis will be sketched in here (Harman 1967; Mulaik 1972).

We begin by discussing component analysis. We continue to consider a $p-$dimensional,x_1, x_2, ..., x_p, system of observable variates. A set of linear composite measures, $q<p$, are defined for the p variable system. That is, we let $c_j = \Sigma W_{ji}x_i + \gamma_j$, $j = 1, \ldots, q$. Ordinarily γ_j is chosen so that the average c_j is zero. These q composites are chosen so as to give the best possible reproduction of the original variables, x_1, x_2, ..., x_p, either in the sense of minimizing squared errors of prediction of the x's from the c's, or maximizing the multiple correlations of the x's on the c's. In this situation, maximizing multiple correlations leads to different procedures than minimizing squared residuals. Reproduction of the x's is not perfect because only q composites are employed and it is hoped that q is much less than p.

Attention focuses not on the coefficients, W_{ij} , which are the compositing weights and hence, by definition, paths from the x's to the c's, but rather on the regression coefficients in $\tilde{x}_i = \Sigma\beta_{ij}c_j + \theta_i$, the usual multiple regression equations for predicting the x's from the components. In point of fact, we usually work with standardized regression coefficients, α_{ij}.

These components can be transformed or, in psychological jargon, rotated, to attain a scientifically meaningful solution. This is because the c_js (j = 1, ..., q) can be replaced by yet another set of composites, $c^*_k = \Sigma\mu_{kj}c_j(k = 1, .X.X., q)$, without loss of additional information. The experimenter must be the ultimate judge of scientific utility. From a strictly mathematical point of view, an infinite number of rotations is possible, but in practice only a few are plausible. A number of procedures exist for attaining simplified structure in the β_{ij}s or α_{ij}s —e.g., VARIMAX (Kaiser 1958; Mulaik 1972). Methods can be employed to guide a rotation to some hypothesis (Meredith 1977).

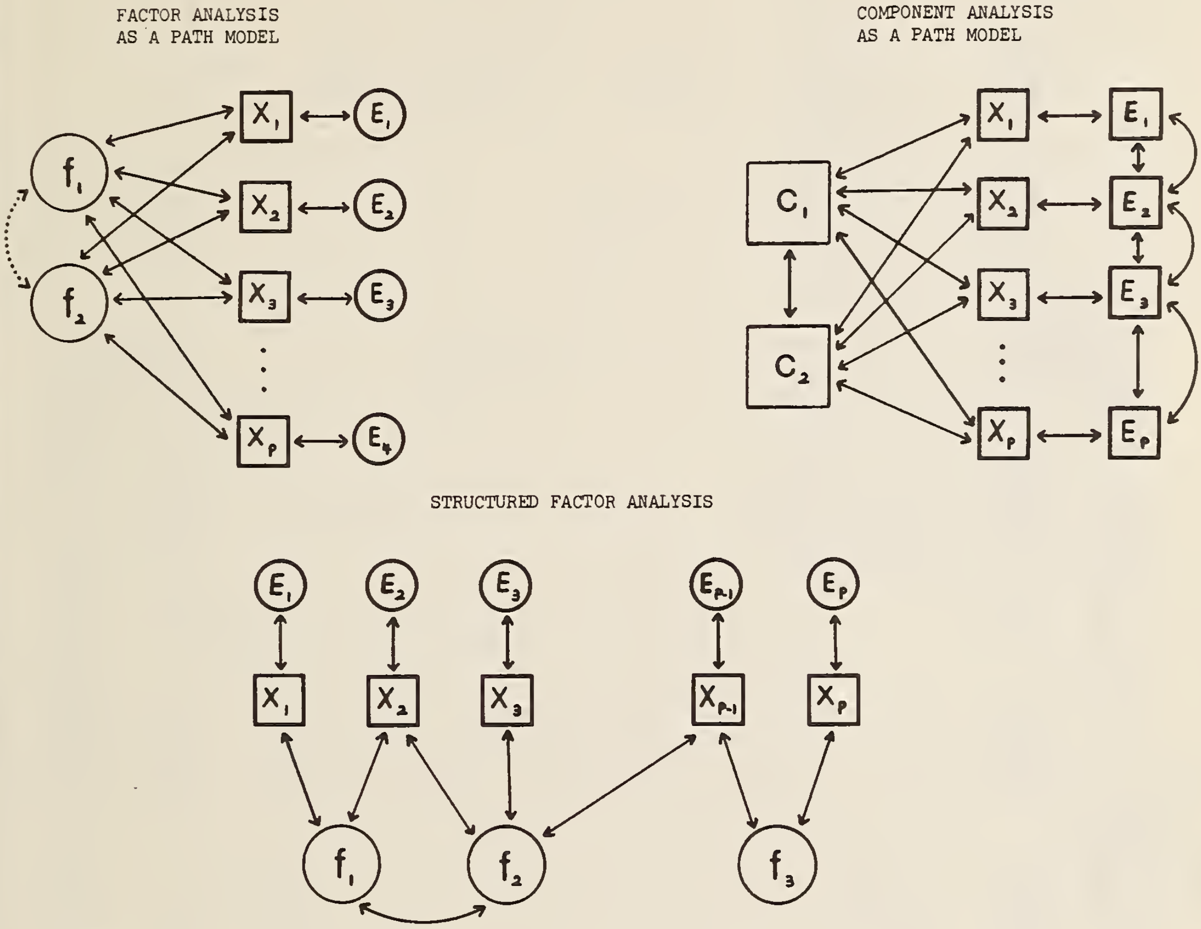
(We observe that components can be defined so as to give the best accounting of mean differences in the x's between groups, or best accounting of main effects and interactions in multivariate analysis of variance, or best accounting of some outside system of additional variables, $y_1, y_2, ..., y_n$. In these cases the component variates are usually referred to as "canonical variates"—Finn 1974; Morrison 1976.)

In factor analysis, in contrast to component analysis, we hypothesize the existence of q, really $q + p$, underlying unobserved variables called q common factors and p specific factors, respectively. Strong structure or models may be imposed in the paths from factors to observable variables. Let f_j denote the jth common factor and e_i the ith specific factor. Attention focuses again on the regression coefficients β_{ij} in the equation $x_i = \Sigma\beta_{ij}f_i + e_i$. Note that the absence of a tilde over the x_i indicates that this is supposed to be an exactly reproductive equation. Again it is customary to look at standardized regression coefficients, α_{ij}, or path coefficients. We note at this juncture that if either the factors, f_j, in factor analysis or components, c_j, in component analysis are chosen to be mutually uncorrelated, the standardized coefficients, α_{ij}, are interpretable as simple correlations.

Both factor and component analysis can be represented as path analytic models as depicted in figure 3. In general, all relations in factor and component analysis are associative, not causal.

It is a remarkable fact that if enough structure to insure identifiability for the f_js is imposed in factor analysis, the path

Figure 3. Path Analytic Models of Factor Analysis and Component Analysis



coefficients from the factors to the observable variables can be calculated. A transformation or rotation problem arises in factor analysis just as it does in component analysis but not in strongly structured factor analysis.

Finally, we take up an example of a factor analysis of a variety of measures of activity, exploration, curiosity, and emotionality in mice (see table 1). The animals are approximately 1,100 individuals derived from a diallele cross of seven inbred strains. Thus, there are 98 genotypes, when sex is taken into account, represented with approximately equal frequency in the 1,100 animals. The measures are four scores from an open-field apparatus, four from an arena (open field with barriers), three from a Y-maze, three from a hole-in-wall test, three from a barrier-climbing apparatus, four from a staircase test, three from a mouse "TV watching" situation, two home cage emergence scores, three from a vertical screen test, and two separate running wheel activity scores. In addition, total urination and total defecations were recorded. Finally, responsiveness to handling was rated. (The apparatus and scores are described in detail in McClearn et al. 1970.)

Table 1. A Factor Analysis of Exploratory Activity

Measure	General Factor	Open Field	Arena	Y-Maze	Hole-in-Wall	Barrier	Staircase	TV	Emergence	Vertical Screen	Wheels	Rearing	Emotionality	?
OFCR	77	41	-11	-11	-10		-10	-10	-11					
OFDW	79	24	-15	-10	-14		-14							-15
OFLW	71	56		-10		-11			-14					
OFCT	54	26							15					-26
ARCT	73		52	-10					-11					
ARLW	72		52	-12	-10		-10		-18					
A113	65		42											-35
ARRG	37		34	-10			-11					38	-20	22
YMM1	90			33	-11		-13				-12			
YMM2	69			59			-10							
YMM3	71			55		-11	-12							
YMRG	36			42								39		34
HWET	82		-12		50									
HWLY	59			11	-61		11							
HWVE	-52				-69		12					26	22	-28
BRXG	76					63								
BRLY	-69	10		11		-64	12							-21
BRRG	56					18						64	20	-30
SCM1	81		-11				55	-10					10	-19
SCM2	68	-11		-15			64						11	-12
SCM3	61					-10	59							
SCTM	17						-71						-11	20

Table 1. A Factor Analysis of Exploratory Activity

Measure	General Factor	Open Field	Arena	Y-Maze	Hole-in-Wall	Barrier	Staircase	TV	Emergence	Vertical Screen	Wheels	Rearing	Emotionality	?
TVFX	84							68						-22
TVNX	70			-11		-10	-13	75						-22
TVTD								55	-11					34
EMRG	52		-12						65			19		20
EMTM	-58			10					-69					-24
VSFE	20						12	-10		-72		13		-23
VSXG	64							-12		86			19	
VSTR	40									89			21	
WHL1	41										76		-20	
WHL2	32										82			
URIN	30											75	32	
BOLI													87	
RSPH	50								11					77

OFCR = open field corner entries
OFDW = open field dark wall entries
OFLW = open field light wall entries
OFCT = open field center entries
ARCT = arena central entries
ARLW = arena light wall entries
All3 = arena squares 1 and 13 entries
ARRG = arena rearing

HWET = hole in wall entries
HWLY = hole in wall latency to first entry
HWVE = hole in wall vicarious entries
BRXG = barrier crossings
BRLY = barrier latency to first crossing
BRRG= barrier rearing
SCM1 = staircase, first minute steps traversed
SCM2 = staircase, second minute steps traversed

TVTD = difference in time spent in "viewing area," TV on vs. TV off
EMRG = rearing during home cage emergence test
EMTM = latency to emerge from home cage
VSFE = vertical screen floor entries
VSXE = vertical screen crossings
VSTR = vertical screen top row entries
WHL1 = wheel revolutions, first day
WHL2 = wheel revolutions, second day

Table 1. A Factor Analysis of Exploratory Activity

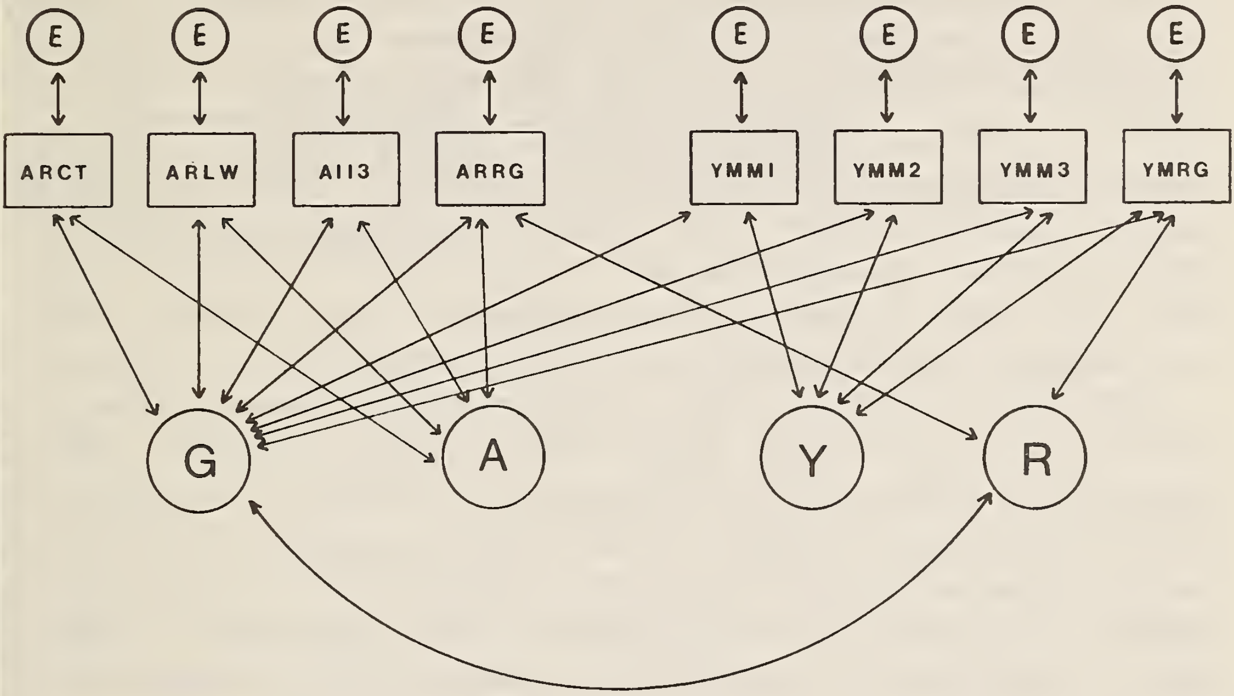
Measure	General Factor	Open Field	Arena	Y-Maze	Hole-in-Wall	Barrier	Staircase	TV	Emergence	Vertical Screen	Wheels	Rearing	Emotionality	?

YMM1 = Y-Maze, first minute entries
YMM2 = Y-maze, second minute entries
YMM3 = Y-maze, third minute entries
YMRG = Y-maze rearing

SCM3 = staircase, third minute steps traversed
SCTM = staircase, time on top steps/number of ascents
TVFX = television "off" crossings
TVNX = television "on" crossings

URIN = number of tests with urination observed
BOLI = number of fecal pellets
RSPH = related responsiveness to handling

Figure 4. Part of a Path Analytic Model for Activity Data



Note that all measures identified in the left-hand column of table 1 whose labels end in RG are rearing scores or measures of vertical exploration almost always accompanied by sniffing and vibrissa twitching. Labels ending in LY or TM denote time or latency; M1, M2, M3 endings designate minute by minute scores. It should be borne in mind that all scores from a given apparatus were obtained from a single test, except for the two wheel scores.

We have a model that presupposes 1 general activity factor; 10 uncorrelated factors, specific to each type of apparatus or test situation and uncorrelated with the general factor; a rearing factor; and 1 or more emotionality factors. The results we present are not final or conclusive. A part of the model depicting the relation of four of these factors to arena and Y-maze scores is depicted in path analytic form in figure 4. The factors are labeled G (general), A (arena specific), Y (Y-maze specific), and R (rearing).

Fourteen common factors provide, in unrotated form, an accurate accounting of the total system of 35 variables. These were transformed by Procrustes methods to the results in table 1 (Meredith 1977). The results are presented in correlational form; that is, the entries in table 1 are standardized regression coefficients and correlation coefficients relating the manifest variables to the common factors (decimals are omitted). Except in the general factor, the large elements correspond to hypothesized nonzero path coefficients. All the rest of the paths are supposed to be zero. To avoid clutter, elements less than 0.10 in absolute value are not recorded. The nonzero coefficients in unexpected locations are an indication of the failure of the model to fit. The 14th factor was chosen to be uncorrelated with the previous 13. The first 12 factors seem to conform to the hypothesized model very well in the sense that the expected zeroes all seem reasonably satisfactory. There is a nearly random pattern of signs in the elements of each column that are expected zeroes. No expected zero exceeds 0.20 in absolute value; hence, less than 4 percent of the variance of any variable could conceivably be accounted for by any factor supposed to be unrelated.

Confirmatory analyses of this model are planned using maximum likelihood and generalized least square methods (Jöreskog 1970; Jöreskog and Goldberger 1972). Because of the diallele cross nature of the data collection, separation of the data into genetic and environmental components is possible, and this structure will be confirmed, if possible, for genetic and environmental effects separately. In addition, we have data for a sample from a random mating heterogeneous population. The model will also be applied to these data.

References

Anderson, T.W. *An Introduction to Multivariate Statistical Analysis.* New York: Wiley, 1958.

Finn, J.D. *A General Model for Multivariate Analysis.* New York: Holt, Rinehart and Winston, 1974.

Harman, H.H. *Modern Factor Analysis.* Chicago: University of Chicago Press, 1967.

Jöreskog, K.G. A general method for the analysis of covariance structures. *Biometrika,* 57:239-251, 1970.

Jöreskog, K.G. A general method for estimating a linear structural equation system. In: Goldberger, H.S., and Duncan, O.P., eds. *Structural Equation Models in the Social Sciences.* New York: Seminar Press, 1973.

Jöreskog, K.G., and Goldberger, A.S. Factor analysis by generalized least squares. *Psychometrika,* 37:243-260, 1972.

Kaiser, H.F. The varimax criterion for analytic rotation in factor analysis. *Psychometrika,* 23:187-200, 1958.

Kerlinger, F.N., and Pedhazur, E.J. *Multiple Regression in Behavioral Research.* New York: Holt, Rinehart and Winston, 1973.

Lazarsfeld, P.F., and Henry, N.W. *Latent Structure Analysis.* New York: Houghton Mifflin, 1968.

McClearn, G.E.; Wilson, J.; and Meredith, W. The use of isogenic and heterogenic mouse stocks in behavioral research. In: Lindzey, G., and Thiessen, D.D., eds. *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype.* New York: Appleton-Century-Crofts, 1970. pp. 3-22.

McNemar, Q. *Psychological Statistics.* New York: Wiley, 1969.

Meredith, W. On weighted procrustes and hyperplane fitting in factor analytic rotation. *Psychometrika,* 42:491-522, 1977.

Morrison, D.F. *Multivariate Statistical Methods.* New York: McGraw-Hill, 1976.

Mulaik, S.A. *The Foundation of Factor Analysis.* New York: McGraw-Hill, 1972.

Namboordi, N.K.; Carter, L.F.; and Blalock, H.M. *Applied Multivariate Analysis and Experimental Designs.* New York: McGraw-Hill, 1975.

Scheffe, H. *The Analysis of Variance.* New York: Wiley, 1959.

Wright, S. *Evolution and the Genetics of Populations.* Vol. I. Chicago: Genetic and Biometric Foundation, 1967.

SECTION IV: REPORTS OF THE TASK GROUPS

Introduction

Gerald E. McClearn

A very large number of phenotypes that would be of utility in alcohol research can be imagined. Indeed, each investigator of alcohol phenomena could probably provide his or her own specifications. However, not every investigator is inclined nor is every laboratory equipped to undertake selective breeding programs. Some consideration is therefore in order concerning those phenotypes that might be expected to be of greatest value to the largest number of investigators and most likely, on the basis of present knowledge, to yield significant insights.

The value of selected lines will likely be directly related to the total amount of information and the different types of information accumulated concerning them. In practice, this will be a function of the number of laboratories making use of the lines. We can therefore imagine a network of cooperating laboratories including both the genetics laboratories that generate them (which, of course, will probably have a major pharmacological program as well) and pharmacological laboratories with the sophistication and interest needed for effective employment of the lines generated by the genetics laboratories. There arise issues of coordination and cooperation among the genetics laboratories and between the genetics laboratories, on the one hand, and the pharmacological laboratories, on the other. To address these issues, the conferees were asked to serve on task groups to review the following topics: (1) the design of selection experiments, (2) identification of phenotypes of choice for alcohol research, (3) coordination of participating genetics laboratories, and (4) coordination of participating pharmacological laboratories. None of these topics can be considered completely independently of the others, and the task group discussions necessarily ranged over the whole spectrum. The results of their deliberations are presented in this section.

The Design of Selection Experiments*

The charge of this task group was to examine options and to make recommendations regarding designs for selection experiments intended for the initial development of animal models for the study of drug abuse and its effects. Within this general framework we have considered two potential restrictions. The first is to maximize the response to selection per unit time. Because the aim of these experiments will be to create experimental material for further study and not to examine the effects of selection per se, the most rapid possible progress is desirable, not only for valid scientific reasons but also for cost efficiency in the initial development of models. The second restriction is the special limitations imposed if the phenotype to be selected results in the death of the measured animal.

The first section of this report deals with general considerations. For a more detailed discussion of the theory on which these recommendations are based, refer to the chapter by DeFries and to the chapter by Roberts.

The choice of an appropriate base population can be of crucial importance when the aim of selective breeding is to develop stocks for future research on the mechanisms underlying the selected phenotype. We recommend use of an established heterogeneous stock, of known genetic origin. As an example, for an experiment with mice an ideal choice would be the HS/Ibg stock which is maintained at the Institute for Behavioral Genetics, University of Colorado, as well as at several other laboratories. There are several advantages to such a stock. The HS is a genetically defined stock, so that information is available concerning the strains that made up the stock and exactly how the stock was constructed and has been maintained. (See the chapter by McClearn for details.) This information is useful for preliminary analyses, as explained below. More important, within the constraints of laboratory stocks, the HS is highly genetically heterogeneous, having been derived from will maximize the ultimate gains from selection. In addition, in cases where a more or less concerted effort is to be made among different laboratories to understand one or a group of related phenotypes, it will be useful to have a readily available common base population from which several selection experiments may be derived.

---

*The members of this task group were J.C. DeFries, Janet S. Hyde, Carol Lynch (Chair), Dennis Petersen, and R.C. Roberts.

Consider alternative populations that have been used in previous selection experiments. (See the chapter by Hyde for a discussion of previous work.) The frequently encountered "randomly bred" animals usually represent stocks of unknown genetic origin and degree of prior inbreeding. In these cases, it is impossible to estimate the extent of heterozygosity, potentially limiting both the usefulness of the stock for ancillary analyses and the gains from selection. A less frequently used alternative is to construct a new stock. This is done most simply by crossing two divergent strains, which limits the genetic variability of the cross to the alleles possessed by the chosen strains. Another possibility is to cross a number of stocks exhibiting phenotypic extremes for the measured trait, such as animals resulting from other selection experiments, if available. Again, this usually involves a lack of specific genetic information about the stocks. However, beyond the genetic characteristics of the stock, several considerations argue against constructing a new stock. Foremost is that of time. Additionally, the stock must be maintained continuously if its constant availability as a reference is desired, and this requires space, time, and money.

Sampling of the base population presents few problems, because it will be largely dictated by design. As an example, consider the case of divergent selection with a control line, using within-family selection. Ideally all three lines (upward, downward, and control) would begin from the same families, the number of families being determined by the number of matings that will be maintained in each line. As a minimum for within-family selection, we suggest eight families per line. Animals from which the control line will be initiated should be chosen first, because their choice should be strictly random, with a male and female chosen from each family. Then the highest scoring male and female and the lowest scoring male and female of animals remaining in each family will be used to initiate upward and downward selection, respectively. This procedure assumes that families sampled will consist of at least three males and three females. Additional replicates would involve a separate sample of families. In some cases where a short-term small experiment is contemplated, it would be advantageous to increase the number of initial families from which the selection is started; e.g., the sample can be doubled simply by taking males from one set of families and females from another. Increasing the base in this way is probably only important for very small experiments (e.g., four pairs per line) where the drift or genetic sampling variance in the first generation could be substantial.

In the opinion of this task group, the optimal design for selection experiments within the aims of this workshop is replicated, bidirectional selection, with control lines. Divergent (in contrast to one-way) selection facilitates the development of animals with the greatest possible phenotypic difference, and one purpose of the creation of animal models is to study the differences between animals with extreme phenotypes.

A contemporaneously bred and measured control line serves several functions. A randomly bred population is the best way to monitor between-generation environmental sources of variation affecting phenotypic expression. Control lines are also useful in the study of mechanism, as, for example, correlated responses in some physiological trait to selection for a behavioral difference. A true genetic correlation, resulting from pleiotropic gene action, will produce a rank ordering of upward, control, and downward selected lines. Control lines that lie outside the range of the selected lines usually signify the effects of random fixation (drift) rather than selective fixation of alleles. Finally, a properly maintained control line is the only way to monitor the effects of inbreeding, as opposed to selection, on the trait being considered, because a control line maintained at the same effective population size as the selected lines will accumulate inbreeding at the same rate, without the effects of selection.

Replication is absolutely essential for the study of the biological bases underlying the response to selection. Typically, selection experiments for behaviors have not involved replication, which has placed severe limitations on conclusions derived from the search for mechanisms in these populations, and may account in part for conflicting results from different laboratories. Inability to detect a correlated response in at least two replicates indicates that the observed correlation may be due to chance association based on fixation of alleles at different loci, rather than at loci influencing the traits in common. Some chance associations are inevitable in small populations and are therefore an unavoidable consequence of selection experiments.

The use of within-family selection, as opposed to mass selection, though in theory less efficient in the short term, is likely to produce better long-term results in the development of animal models. Although the initial response to selection will be reduced, the limits of the response may be increased, and several recent behavioral selection experiments have shown good empirical responses within a few generations using within-family selection. This type of selection is the best way to minimize inbreeding, because the restriction that each family must contribute one male and one female to the next generation doubles the effective population size and consequently halves the percentage increase in homozygosity per generation. Avoidance of inbreeding is important not only because it reduces the genetic variance on which selection can act (thereby lowering the limit of the response) but also because the traits most affected by inbreeding are those connected with fertility. Extensive inbreeding will result in the loss of lines due to inability to breed. Within-family selection also controls for maternal effects, which are nongenetic souces of variation and may hinder selection. In mammals, maternal effects are ubiquitous and sometimes subtle, and the restriction imposed by within-family selection insures that such effects will not confound the selection, because "good" mothers contribute equally with "poor" mothers.

Because the presence of additive genetic variance for the measured phenotype is crucial for a response to selection, it is useful to have some information about genetic effects on the chosen phenotype before the actual selection program is started. As a minimum, the trait must have a nonzero narrowsense heritability. The fastest, easiest, and, therefore, least expensive way to demonstrate the presence of additive genetic variance is to screen inbred strains for significant differences. Although the resulting estimate of extent of genetic difference cannot be used to predict accurately the expected response to selection, it will demonstrate that a response should occur, especially if the strains measured are to some extent representative of the base population for the selection experiment. This further argues for choosing a base population of known genetic origin. Because the most efficient way (in terms of number of animals tested) to obtain a precise heritability estimate that is valid for a genetically heterogeneous population is from the response to selection, it is not worthwhile to attempt to get such an estimate from the base population itself (e.g., by parent-offspring regression), if the primary aim of obtaining the estimate is to predict the response to selection.

In order to produce a large and consistent phenotypic difference between selected lines, selective breeding should be continued for at least 10 generations. This is an empirical estimate, based on average responses from previous selection experiments and represents a minimum of 3 years time, depending on the age at which animals are tested and the length of time required for testing. There is no theoretical basis for predicting the number of generations over which a good response to selection will be maintained, except that traits with high heritabilities which are influenced by genes at few loci will show a rapid response and an early plateau. Because the aim of the selection is to produce animals of widely divergent phenotypes, the selection program should be continued as long as a reasonable response is being realized.

The above discussion has been based on the optimal recommended design, assuming that individual animals can be measured and bred and that the measurements are relatively straightforward so that sufficient prior genetic information can be obtained from inbred strains. A major complication in the design and execution of selection experiments arises when the phenotype to be selected results in the death of the measured animal.

There are a variety of alternatives to individual selection, all of which vastly increase the demands for space if a constant effective population size is to be maintained. The most obvious of the alternatives is progeny testing. In this case, large numbers of animals are mated, and their offspring are killed and measured. Those parents with the highest (in the case of upward selection) family means are selected and bred again to produce the next generation. This nearly doubles the time between production of selected generations.

A converse approach is retrospective selection. Again, large numbers of animals are mated, but in this case the parents are killed and measured. The offspring from the parents that are then selected retrospectively on the basis of their phenotypes are saved for breeding, and the rest of the families are eliminated. This requires less time than progeny testing because the parents do not have to be bred again, but it decreases the selection differential, because, since mating has already occurred, the parent average must be used, rather than the individual parent scores. In practice, this would probably also be true of progeny testing.

A third alternative is sib selection. Animals that potentially will be bred are saved (e.g., a male and female from each of many families), and the remaining siblings are killed and measured. Similar to progeny testing, animals are selected on the basis of their family means; in this case the means of their siblings, rather than their offspring. Although under most circumstances, sib selection is genetically less efficient than progeny testing or retrospective selection (because sibs do not predict additive genetic values as accurately as offspring do), it saves time because the selected animals can be bred to produce the next generation as soon as they are old enough. Therefore, to balance genetic efficiency with time efficiency, sib selection is preferred, and progeny testing least desirable. It should be emphasized that all of these methods are slower and require considerably more space than individual selection, and are therefore much more costly. For these reasons, it is probably worth investing time in a preliminary search for a nondestructive phenotype that is genetically correlated to the trait of interest, e.g., select for a behavioral response, then examine the physiological basis for this response in later experiments.

Another possible complication occurs when it is considered desirable to select for more than one trait simultaneously. Such a contingency can again be handled in several ways. Procedures that require minimum prior genetic knowledge about the multiple traits include tandem selection, in which each trait is selected for in turn. This requires substantial additional time, essentially adding an extra generation interval for each trait beyond the first that is selected. A second procedure is independent culling, in which animals to be bred must reach some minimum standard on all the traits being considered. However, the best method for maximum response to selection for multiple traits is referred to as index selection, in which all the measured traits are combined into a single numerical index, appropriately weighted by their heritabilities and the genetic correlations among them. In practice, however, construction of such an index requires precise prior information about the phenotypic and genetic variances of the traits, as well as the phenotypic and genetic correlations among the traits. Because some of these estimates are difficult to obtain, even with large numbers of animals, multiple trait selection is considerably more difficult and consequently less efficient than individual trait selection.

It may be useful to distinguish between multiple traits and a complex trait with multiple components. For example, a character such as "addiction" could be defined as a series of single measurable factors (dependency, tolerance, etc.), or could be redefined statistically into a single measurement derived from multivariate analysis. (See the chapter by Meredith for further discussion.) As in index selection, construction of the complex trait requires preliminary analysis on the base population, but the complex trait can be constructed without knowledge of the genetic variances and covariances of the individual factors. Once a complex trait has been designed, selection for that trait reduces to individual trait selection.

Finally, the following suggestions are aimed at maximizing the gains per unit time from a selection experiment: (1) The added time usually obviates any advantage in increasing family size by measuring second litters. (2) Animals should be tested as young as possible to minimize the generation interval. (3) Every attempt should be made to select for a trait that does not require killing the animal, even if extensive preliminary investigations are required. (4) If multiple traits are to be selected, construction of a complex trait using the procedures of multivariate analysis is preferable to the construction of an index for multiple trait selection. (5) Although it should go without saying, strict environmental control during the production and testing of experimental animals is especially important during a selection experiment, because any random environmental variation or error of measurement affecting the chosen phenotype will reduce the heritability and, consequently, the rapidity of the response to selection.

It should be obvious that designing selection experiments can be complicated, especially if the phenotypes are destructive or complex. Investigators contemplating such experiments are urged to seek advice from statistical geneticists both before and during the course of the experiment, to insure optimal results in the production of useful animal models.

Phenotypes To Be Selected for Breeding Purposes in Alcohol Research*

The task group charged with recommending the phenotypes that would be important in alcohol research initiated its deliberations by agreeing on the following assumptions:

1. Based on the positive results that have been achieved during the last decade using inbred strains of mice in cancer research and the current usefulness of the limited number of strains which have been selected for differing sensitivity to ethanol, we assume that further application of selective breeding could be beneficial in developing experimental subjects for alcohol research.
2. Phenotypes should be selected on the basis of pharmacological responses to alcohol. We assume that the fundamental information gained will ultimately become relevant to human alcoholism.

Prior to the discussion of valuable phenotypes, the task group had to arrive at a consensus regarding the definition of certain terms to be used in describing the phenotypes. The following definitions are meant to be used in the specific context of this report and should not be taken as the only means of identifying the various phenomena related to the pharmacology of ethanol.

1. *Metabolism*: the rate of disappearance of ethanol or acetaldehyde from the organism.
2. *Preference*: consumption of ethanol in the presence of alternative fluids.
3. *Initial sensitivity to ethanol*: physiological, biochemical, or behavioral responses to ethanol in the naive animal (an animal not previously exposed to ethanol).

---

*The members of the task group were Allan Collins, C.J.P. Eriksson, Ryoko Kakihana, William Meredith, Boris Tabakoff (Chair), and Ronald Thurman (Rapporteur).

4. *Acute tolerance*: diminished effect of ethanol on physiological, biochemical, or behavioral functioning measured on the descending limb of the blood or brain ethanol curve, compared with effect of the same concentration of ethanol on the ascending limb of the curve in an animal given a single dose of ethanol.

5. *Developed tolerance*: diminished effect of a particular dose of ethanol on physiological, biochemical, or behavioral systems following repeated exposures to ethanol.

6. *Psychological dependence*: maintenance of operant behavior with ethanol as the positive reinforcer.

7. *Physical dependence*: operationally defined as the appearance of a withdrawal reaction after the elimination of ethanol from an organism following chronic administration of ethanol.

8. *Pathology*: pathologic changes in physiological, behavioral, or biochemical parameters following chronic exposure to ethanol.

Selection of Specific Phenotypes

In its deliberation on specific phenotypes that may be valuable in alcohol research, the group placed heavy emphasis on phenotypes that could be ascertained without having to sacrifice the animal and on effects that could be determined simply and rapidly. Such criteria would aid a more rapid and less expensive development of selected lines of animals. The selection criteria should also generate lines of animals that would differ significantly in various parameters of interest to alcohol researchers at present and in the foreseeable future. After the lines were established, animals could then be used for studies of the underlying physiological or biochemical mechanisms producing the phenotypes for which the animals were selected. Thus, although little explanation will be particular phenotypes in clarifying certain major problems currently under study in alcohol research, the utilities of these phenotypes should be self-evident to researchers in a particular area. Superscript symbols in the following sections are to be interpreted as follows:* represents a phenotype on which some information is available in selected lines of animals; †indicates that lines have already been selected for this particular phenotype; ‡indicates that more than one selection study has utilized this phenotype as the basis for selection.

Metabolism

The task group considered breeding for specific enzymes related to ethanol and aldehyde elimination but ultimately rejected this idea because of the utility of the models described below. By use of proper genetic-biochemical correlation analysis, positive information with respect to mechanisms should be forthcoming without breeding for specific enzyme systems.

Phenotypes: (1) elimination of a low dose of ethanol* (Heston et al. 1974); (b) elimination of a high dose of ethanol and increases in ethanol elimination after high doses of ethanol* (Heston et al. 1974; Thurman et al. 1979); (c) acetaldehyde elimination* (C. Eriksson 1973).

Initial Sensitivity

The group felt that analysis of sensitivity to ethanol had to include consideration of both the stimulant (disinhibiting) and depressant effects of ethanol. Therefore, phenotypes should reflect differences in response to both hypnotic and subhypnotic (behaviorally activating) doses of ethanol.

In the following list, the first four phenotypes are related to hypnotic doses, while the fifth is a measure of subhypnotic effects: (1) loss of righting reflex† (McClearn and Kakihana 1973); Erwin et al. 1976); (b) temperature changes; (c) respiration; (d) tilting plane (motor activities)† (Lester et al. 1977; Rusi et al. 1977); (e) locomotor activities* (Lester et al. 1977).

Acute and Developed Tolerance to Alcohol

The group felt it would be useful to develop phenotypes that would differ in rate of acquisition, disappearance, and extent of acute and developed tolerance following ethanol administration. The same five phenotypes listed under "Initial Sensitivity" should be employed in developing such lines: (a) loss of righting reflex; (b) temperature; (c) respiration; (d) tilting plane (motor activities)*; (e) locomotor activities*.

Preference‡

The most widely studied phenotype in ethanol research is preference for ethanol. A wide body of information on preference or lack of preference for ethanol in a variety of inbred strains and selected lines of animals has convinced the task group that a preference for alcohol is clearly a phenotype of importance which can be obtained through selective breeding procedures. The phenotype should be defined as it has been in previous studies (K. Eriksson 1968; Lumeng et al. 1977) as the selection of ethanol in the presence of alternate fluids.

Psychological Dependence

Because of the difficulty in accurately assessing psychological dependence in experimental animals, the task group felt that a phenotype of this nature, while desirable, would be difficult to define operationally. The general consensus was that operant behavior paradigms could, however, be used in defining this phenotype.

Physical Dependence

The group felt that phenotypes that differed in locomotor activity changes and indicators of CNS hyperexcitability (seizure susceptibility) after chronic treatment with ethanol might be valuable for ascertaining some of the mechanisms responsible for the development of physical dependence. However, Goldstein's report (1975) and previous experiments demonstrated that one must cautiously approach the problem of developing such a phenotype because one does not know whether one is breeding for acquisition of physical dependence or expression of characteristics of the withdrawal reaction (i.e., sensitivity to convulsions). A phenotype that could dissociate or give information on both of these parameters would, however, be highly desirable.

Pathology

Abuse of alcohol is clearly associated with many pathologies in animals and humans. However, the lack of equivalent pathologies in animals and humans makes the selection of particular phenotypes in animals of questionable value. For example, cirrhosis is a major health problem, but there is no unequivocal animal model of it at this time. The task group did feel that animals which differ in sensitivity to ethanol's effects in producing lesions in the central nervous system and learning deficits could be developed through selective breeding and beneficially utilized. The group discussed the fetal alcohol syndrome and various alcohol myopathies. Phenotypes related to these phenomena were considered to be desirable but difficult to develop or to have low priority.

Conclusions

The major areas that at present are amenable to studies using selective breeding techniques are those concerned with the metabolism of and initial sensitivity to ethanol and areas concerned with acute and developed tolerance to this drug. That several lines of animals have already been selected for preference to ethanol attests to the ability to select for this phenotype. The other phenomena which accompany either the acute or chronic administration of ethanol as listed above may prove more difficult to define in terms of a phenotype which is easily distinguished and for which selection studies could be initiated. This caveat, however, should not prevent an active search for definitive phenotypes reflecting such phenomena as physical dependence, psychological dependence, etc. Even in these hard-to-define areas, selective breeding would eventually be of immense value.

References

Eriksson, C.J.P. Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochem Pharmacol*, 22:2283-2292, 1973.

Eriksson, K. Genetic selection for voluntary alcohol consumption in the albino rat. *Science*, 159:739-741, 1968.

Erwin, V.G.; Heston, W.D.W.; McClearn, G.E.; and Deitrich, R.A. Effect of hypnotics on mice genetically selected for sensitivity to ethanol. *Pharmacol Biochem Behav*, 4:679-683, 1976.

Goldstein, D.B. Physical dependence on alcohol in mice. *Fed Proc*, 34:1953-1961, 1975.

Heston, W.D.W.; Erwin, V.G.; Anderson, S.M.; and Robbins, H. A comparison of the effects of alcohol on mice selectively bred for differences in ethanol sleep-time. *Life Sci*, 14:365-370, 1974.

Lester, D.; Lin, G.W.-J.; Anandam, N.; Riley, E.P.; Worsham, E.D.; and Freed, E.X. Selective breeding of rats for differences in reactivity to alcohol: An approach to an animal model of alcoholism. IV. Some behavioral and chemical measures. In: Thurman, R.G.; Williamson, J.R.; Drott, H.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems: Intermediary Metabolism and Neurochemistry.* Vol. III. New York: Academic Press, 1977. pp. 635-642.

Lumeng, L.; Hawkins, T.D.; and Li, T.-K. New strains of rats with alcohol preference and nonpreference. In: Thurman, R.G.; Williamson, J.R.; Drott, H.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems: Intermediary Metabolism and Neurochemistry.* Vol. III. New York: Academic Press, 1977, pp. 537-544.

McClearn, G.E., and Kakihana, R. Selective breeding for ethanol sensitivity in mice. (Abstr.) *Behav Genet,* 3:409-410, 1973.

Rusi, M.; Eriksson, K.; and Mäki, J. Genetic differences in the susceptibility to acute ethanol intoxication in selected rat strains. In: Gross, M.M., ed. *Alcohol Intoxication and Withdrawal.* Vol. IIIA. New York: Plenum Press, 1977. pp. 97-109.

Thurman, R.G.; Yuki, T.; Bleyman, M.; and Wendell, G. The adaptive increase in ethanol metabolism due to pretreatment with ethanol: A rapid phenomenon. *Drug Alcohol Depend,* 4:119-129, 1979.

Coordination of Participating Pharmacological Laboratories*

The task group was faced with a number of items for consideration, many of which are listed below.

1. How may we insure genetic integrity when the animal models are widely distributed? Problems may be particularly severe in new laboratories that lack experience in maintaining selected lines.

2. When in the development of line differences will it be appropriate to begin ancillary studies?

3. How can exchange of data, animals, and personnel (faculty and students) among cooperating laboratories be made efficient? When few animals of a given line are available, do we risk exchanging animals or do we exchange people? Is there a possibility of applying for funds to support exchange?

4. It seems likely that demand will exceed supply of the selected animals. If so, choices will have to be made. How shall priorities be assigned? Should allocation depend on use rate and level of research productivity? Should priorities be assigned so as to maximize the types of data that will be accumulated? Should particular areas, i.e., receptors, enzyme differences, etc., have higher priorities than other areas?

5. Who should decide on the priorities, the generating laboratory or an advisory board?

From initial discussions about "where to start" it soon became evident that availability of animals and phenotypes to be used were the questions that had to be answered first. Regardless of which laboratories would participate in the screening, using animals from an ongoing supplier would easily exhaust the supply. Second, the phenotypes to be used should be decided. Since breeding for selected phenotypes takes time, it was suggested that existing breeders be funded to expand breeding and supply capacity. Ongoing breeders who were in an active process of pharmacological and biochemical research were more attractive as far as capabilities were concerned. It was felt that these laboratories had more of an invested interest and would provide a better quality control assurance.

---

*The members of the task group were Jules Cass, Richard Deitrich, Carlton Erickson, Dora Goldstein, Richard Marcus, Christine Melchior, and David Ross (Chair).

The task group discussed whether a pharmacology advisory committee was needed. Some of the committee's duties would cover quality control for animal handling between supplier and laboratory or between laboratories when strains are transferred. The committee might also handle information exchange in the form of a newsletter circulated among interested laboratories. One central laboratory might act as a coordinator for receiving information. Since supply of strains may be limited, the committee might establish priorities for pharmacological testing so that some laboratories would not duplicate the work of others. The committee might have input for possible design of program project grant applications or other similar types of funding.

Close contact between the pharmacological laboratories and the breeder-supplier laboratories is of major importance if research and breeding efforts are not to be wasted. Information gained by the cooperation and efforts of the breeder-supplier and cooperating pharmacological laboratories will be useful to new investigators who may want to enter this field of pharmacogenetics. We discussed the possibility of preparing a handbook on pharmacogenetics to include questions to be asked when considering establishment of breeding laboratories.

Funding sources and possible areas of interest to granting institutions were discussed. Agencies most likely to be interested might include NIAAA, the NIDA Animal Resources Branch, and the Biotechnology Branch of the U.S. Department of Health and Human Services. Also considered was the overall atttractiveness of a pharmacogenetic model for studies other than drug research (such as hypertension, kidney diseases, etc.).

Coordination of Participating Genetics Laboratories*

This task group discussed the following topics: exchange of information on animal models and selected stocks useful in substance abuse research; availability and production of existing selected stocks such as those developed by Erickson, Lester, Li, and McClearn and described previously in this conference report; development, production, and availability of new selected stocks for substance abuse research; and the conditions under which existing and new selected stocks might be obtained from a breeding laboratory by other potential users.

With respect to information exchange, the task group suggested that there should be a periodic bibliography of articles dealing with genetics, selected stocks, and substances of abuse, especially alcohol; Lester offered to initiate and to distribute such a bibliography through the Rutgers Alcohol Research Center. The group also recommended that there should be a newsletter concerning selected stocks of laboratory animals. This newsletter would be similar to the *Mouse Newsletter* and to the recently established *Rat Newsletter*. Such a newsletter might provide information on the origin and breeding history of the selected stocks, the genetics of the selected stocks, the phenotypic measures used with the selected stocks, and other pertinent details. The availability and conditions for obtaining animals from the selected stock by potential users might also be described in the newsletter. It was also recommended that the newsletter should be prepared and distributed, at least initially, on an annual basis. The group also suggested that funds be obtained for publishing this newsletter and that someone be found to initiate and to organize the newsletter.

Next, the task group discussed the topic of supplying animals of selected stock(s) from the breeding laboratories to other potential users. Erickson, Lester, Li, and McClearn maintain selected stocks of animals bred for phenotypes useful in alcohol research, and each has made animals from their selected stock(s) available to other genetic and/or pharmacological laboratories. However, because these breeding laboratories are not large-scale commercial suppliers of animals, only a limited number of animals from any of these selected stocks has been available for use by other investigators. The members of the group felt that the breeding laboratories would require an increase in funds and support, perhaps from NIAAA, if they were to expand production of their selected stock(s) to meet a substantial demand for them by potential users. As an alternative, it was suggested that arrangements might be made to have a commercial breeder supply animals from these selected stock(s) to potential users. Of course, the commercial breeder would need to continue the selection pressure in each generation and to test animals on the desired phenotype.

---

*The members of the task group were Kalervo Eriksson, Gary Horowitz, David Lester, T.-K. Li, Stephen Maxson (Chair), and James Wilson.

None of the members of the task group planned to initiate new selective breeding programs to develop additional stocks of animals usable in substance abuse research. However, they felt that selective breeding for new stocks would be initiated by those desiring animals genetically divergent on some novel phenotype(s). In this context, the members of the group recommended that those initiating new selective breeding programs should use a heterogeneous stock of animals as the foundation population. The development, use, and advantages of a heterogeneous stock for a selective breeding program have been described by McClearn et al. (1970) and are also discussed in the report from the task group on the design of selection experiments. At least one heterogeneous stock exists for mice, and we recommend that one be developed for rats. Inbred strains of rats exist that could be used in establishing the heterogeneous stock (Jay 1963). The group also recommended that funds be sought from NIAAA to support the establishment and maintenance of this heterogeneous stock.

With regard to the experimental design of any new selective breeding program, the members of this group were basically in agreement with the recommendation of the task group on the design of selection experiments that, whenever possible, the selective breeding program should be designed not only to establish new stocks of divergent phenotypes but also to permit elegant and sophisticated genetic analyses of the phenotypes, as well as the study of potentially correlated phenotypes. However, our task group also recognized that practical as well as theoretical considerations may dictate the design of a selective breeding program. Among those practical considerations are funds, space, institutional rules, and research goals.

This task group concluded its meeting with a discussion of the conditions under which existing and new selected stocks might be obtained from a breeding laboratory by potential users. The group suggested that the following conditions might be required of a potential user by one or more breeding laboratories: furnish a protocol of the intended research using the selected stocks, bear the expense of obtaining the animals, cite the source of the animals in oral presentations and in publications, report relevant results to those supplying the selected stocks, and refrain from breeding sublines of the provided stocks that are three or four generations removed from the selected stock itself. The task group members felt that the specific conditions for obtaining mice should be decided by each supplier and published in the newsletter recommended previously.

References

Jay, G.E., Jr. Genetic strains and stocks. In: Burdette, W.J., ed. *Methodology in Mammalian Genetics.* San Francisco: Holden-Day, 1963. pp. 83-123.

McClearn, G.E.; Wilson, J.R.; and Meredith, J.E. The use of isogenic and heterogenic mouse stocks in behavioral research. In: Lindzey, G., and Thiessen, D.D., eds. *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype.* New York: Appleton-Century-Crofts, 1970. pp. 3-22.

Concluding Comment

The general theme of this conference has been that genetic control in general and specifically the use of selectively bred lines will prove to be of immense value in alcohol research. If this is true, an appreciation of the advantages of using genetically specified animals will gradually diffuse throughout the pharmacological community, and the percentage of reported studies using inbred strains or selected lines will increase year by year in the journals. It is not only the pharmacogenetic zealot who can foresee the day when genetic control will be as routinely expected as control of pH or of dosage level, in just such a manner as genetic control has come to be routine in modern cancer research, for example. It is the hope of the organizers of this conference that it may have served the purpose of accelerating the rate of diffusion.

Gerald E. McClearn

366-072 0 - 82 - 20 : QL 3

Glossary

Allele one of the alternative forms of a gene at a particular locus.

Breeding value value of an individual estimated from the mean of its progeny; value predicted for a genotype from least squares fit of relationship between genotype and genotypic value.

Dominance situation in which the heterozygote has the same phenotype as one of the homozygotes.

Drift change of allelic frequencies in a small breeding population due to chance fluctuations.

Environmental deviation difference between the phenotypic value and the genotypic value.

Gene the particulate unit of heredity; a sequence of DNA that codes one polypeptide chain or one RNA molecule.

Gene pool the total of all alleles in a breeding population.

Genotype the genetic constitution with respect to a particular locus or loci.

Genotypic value the genotypic value of a genotype as estimated by the average phenotypic value of all individuals in a population with that genotype.

Heritability, broad sense also termed "degree of genetic determination"; the ratio of genetic variance to total phenotypic variance.

Heritability, narrow sense the ratio of additive genetic variance to total phenotypic variance.

Heterozygote an individual possessing unlike alleles at a particular locus.

Heterozygous possessing unlike alleles at a particular locus.

Homozygote an individual possessing like alleles at a particular locus.

Homozygous possessing like alleles at a particular locus.

Inbreeding mating of related individuals.

Linkage association of alleles in transmission due to their loci being on the same chromosome.

Locus a fixed position in a chromosome that may be occupied by one of the alleles of a particular gene.

Overdominance situation in which the heterozygote phenotype falls outside the range of the homozygote phenotypes.

Phenotype observed or measured characteristic.

Polygenic influenced by more than one locus.

Progeny test selective breeding in which progeny phenotypes are included in selection criterion for individuals.

Repulsion situation in which an individual heterozygous at two linked loci has one dominant and one recessive allele on each chromosome.

Selection differential reproduction.

Selection differential difference between the mean of individuals selected to be parents of the next generation and the mean of the total population from which they were selected.

Selection, family selective breeding in which families are accepted or rejected as units.

Selection, independent culling selective breeding for two phenotypes simultaneously.

Selection, index selective breeding in which the selection criterion is a composite of several phenotypes.

Selection, individual see mass selection.

Selection, intensity of selection differential expressed in standard deviation units.

Selection, mass selective breeding in which the sole criterion of a selection is the phenotype of the individual (same as individual selection).

Selection, response to difference between the mean of the progeny of selected parents and the mean of the parental generation.

Selection, sib selective breeding in which individuals are selected or rejected for breeding on the basis of their sibs' phenotypes.

Selection, tandem selective breeding for two phenotypes in sequence.

Selection, within-family selective breeding in which the highest (lowest) individuals within each family are selected to propagate the high (low) line.

Selective breeding choosing for breeding only individuals with certain phenotypes.

Variance, additive genetic variance associated with the average phenotypic differences of genotypes with 0, 1 or 2 copies of a particular allele.

Variance, dominance genetic variance associated with the deviation of a heterozygote from the mean of the two homozygotes.

Variance, environmental all phenotypic variance not attributable to genetic sources.

Variance, epistatic genetic variance associated with the difference in effect of an allelic substitution in the presence of different genotypes at another locus.

Variance, genetic variance attributable to additive, dominance, and epistatic effects of genetic differences among individuals in a population.

Variance, phenotypic the variance of the measured characteristic.

Index

A

AA (*See* Alko, Alcohol strain) 87
Accumulated inbreeding 50
  (*See* Response to selection) 50
Acetaldehyde
  in AA and ANA rats 128, 134
  effect on alcohol consumption 135
  ethanol elimination tests 236
Acetylcholine 180
Acoustic priming studies 68
Additive genetic value 153
  heritability equation 41
  single-locus case 26
Additive genetic variances 274
Adrenocortical response
  in LS and SS mice 167
Aggressiveness studies
  in female mice 67
  in male mice 64
AKR mice
  morphine studies 216
Alanine
  in P and NP rat brain 180
Alcohol aversion
  acetaldehyde-induced 134
Alcohol dehydrogenase
  in LA and MA rats 197
  in AA and ANA rats 128
  in mice 129, 235
Alcohol dependence syndrome
  animal models 238
    rats 172, 173
  in humans 171, 172
Alcohol deprivation effect
  in AA and ANA rats 139
Alcoholism (*See* Alcohol Dependence Syndrome)
Alcohol Nonpreferring rats
  consumption scores 113, 174
  ethanol elimination rate 176
  selective breeding 173
  sleep time studies 181
Alcohol Non-Tolerant strain
  rat studies 88
    selection response 114
  voluntary ethanol consumption
    in male and female rats 136
Alcohol preference score (*See also* Phenotypic measures) 90
  ketone body excretion 126
Alcohol-Preferring rats
  consumption scores 113, 174
  ethanol elimination rate 176
  selective breeding 173
  sleep time studies 181
  tolerance 186
Alcohol-related traits 87
Alcohol sensitivity tests
  open-field 96, 99
  Rotarod 96, 98
  tilting plane 96
Alcohol tolerant strain
  rat studies 88, 113, 136
Alcohol withdrawal
  in LS and SS mice 165, 169
  in P rats 185
Aldehyde dehydrogenase 133
Aldehyde dehydrogenase inhibitor 125

Alko, Alcohol strain (AA) 88
  acetaldehyde level 128, 134
  alcohol dehydrogenase level 129
  alcohol deprivation effect 139
  alcohol drinking behaviors 120
  brain serotonin 127
  food utilization 124
  open-field activity 122-123, 137
  purpose of selection 111
  sleep time studies 136
  vasopressin excretion 122-123, 137
Alko, Non-Alcohol strain (ANA) 88
  acetaldehyde level 128, 134
  alcohol dehydrogenase level 129
  alcohol deprivation effect 139
  alcohol drinking behavior 120
  brain serotonin 127
  food utilization 124
  open-field activity 122-123, 137
  purpose of selection 111
  salt-seeking behavior 122-123, 139
  sleep time studies 136
  urinary excretion 122-123, 137
Allelic drift 82
ANA (*See* Alko, Non-Alcohol strain)
Analgesia 213, 215, 219
Antabuse (*See* Disulfiram)
Anticonvulsant action 169
ANT (*See* Alcohol Non-Tolerant strain) 88
Apomorphine 167
Artificial selection 54-55
Aspartate 180
Astra diet
  in rat studies 125
  containing cyanamide 129
AT (*See* Alcohol Tolerant strain) 88
Audiogenic seizure study 64

B

BALB mice
  effect of morphine 212, 216
  open-field test 12
Barbital
  tilting plane performance 136
  tolerance 88, 111
Basal metabolic rate 126
Base population 269-270
Behavioral selection studies
  in LS and SS mice 162
  in chickens 72
  in Drosophila 72
  in Japanese quail 72
Between-Family Selection (*See* Selection methods) 46-47
Biscuculline 167
Bidirectional selection
  early studies of 60
  experimental design 273
Blood acetaldehyde levels
  in AT and ANT rat strains 103
  in P and NP rats 178
Blood alcohol levels
  in AT and ANT rat strains 103
Blood pressure studies 69
Body temperature
  tolerance testing 240
Body-weight difference 124-125
Brain dopamine concentration 128
Brain serotonin
  plasma tryptophan 127
Brain serotonin concentration
  chronic ethanol treatment 127
  food intake 127
Brain weight studies 69
Breeding value (*See* Additive genetic value)
Broadhurst
  early selection studies 63
Butanol 163
Butanol, tertiary
  dose response curves

    - in LS and SS mice 163
  - experiments 237
  - tilting plane performance
    - in AA and ANA rats 136
  - tolerance in rats 88, 111

C

- C3H mice 216
- C57BL mice
  - alcohol consumption
    - activity depressant 147
  - alcohol dehydrogenase 131
  - allelic frequency 24
  - breeding studies 63, 83
  - effect of morphine 212, 216
  - morphine-saccharin solution 224
  - open-field test 12
  - tolerance development 243
  - withdrawal seizures 244
- Caloric intake
  - in AT and ANT rats 109
  - in P rats 185
  - regulation of alcohol consumption 124
- Catecholamines 237
  - acetaldehyde-induced release 132
  - alcohol preference 134
  - morphine administration 218
- Causal network hypothesis 250
- CBA mice 131
- Central nervous system 241
  - sensitivity to ethanol
    - in LA and MA rats 196
    - in LS and SS mice 161, 163
    - in P and NP rats 180
- Chemotaxis
  - in Drosophila 73
- Chlorophenylalanine
  - brain serotonin
    - in rats 128
    - in mice 218
- Clonidine 168
- Component analysis 255-256
- Correlated responses (*See* Response to selection) 81-82
- Corticosteroids 167

D

- Dark cycle consumption 179, 186
- DBA mice
  - alcohol consumption 147
  - alcohol dehydrogenase level 131
  - effect of morphine 212, 216, 224
  - low alcohol preference 83
  - tolerance development 243
  - withdrawal seizures 244
- Deckard acoustic studies 68
- DeFries open-field studies 66
- Diethyl ether response curves 163
- Differential fertility
  - genetic variation 37
  - selection process 45
- Directional dominance 153
- Disulfiram 131
- Divergent selection 45
- Dominance deviation
  - polygenic case 27
  - single-locus case 27
- Dopamine 218
- Double testing 235
- Dowel test 238
- Drinking behavior
  - alcohol consumption tests 90
  - in rats
    - relevance to human drinking 112
    - sleeping-time test 88
    - tilting-plane test 88
- Drosophila 73
- "Dummy Coded" variables 252

E

- Environmental influences
  - common environment 48
  - maternal effects 48
- Ethanol elimination rate
  - ADH testing in mice 235
  - in AT and ANT rats 104
  - in P and NP rats 176
- Ethanol oxidation 126

F

- FT procedure (*See* Fall time) 148
- Factor analysis 255
- Fall-time response
  - in mice 148
  - in SS rats 153

Family selection (*See* Between-Family selection) 46-47
Fatty liver 236
Fenfluoramine 128
Fertility index 94
Flurothyl
  in LS and SS mice 167, 168
  seizure susceptibility 244
Food utilization efficiency index 124, 126
Foundation population
  heterogeneous stock 286
Frings seizure study 64

G

Gamma aminobutyric acid (GABA) 165-166
Gammabutyrolacetone
  in LS and SS mice 166
  in P and NP rats 180
Genetic correlation 31
Genetic dissociation 219
Genetic variance
  additive 26, 41, 153, 272
  nonadditive 58
Genotypic frequencies 24, 25
Generation interval 40
Gene substitution 26
Genetic asymmetries (*See also* Response to selection) 51
Geotaxis 72-73
Glucocorticoid response 237
Glutamic acid
  open-field activity 22
  in P and NP rat brain 180
Glutamic acid decarboxylase (GAD) 22
Glycine 180
Goldstein-Pal procedure 206

H

Habituation period 121
Hall studies 62
Haloperidol 167
Hebb-Williams rat maze 61
Hepatic metabolism 179
Heritability
  definition 4, 13
  genotype/phenotype ratio 40
    less than unity 6
    small relation 7
    unity 5
  regression equation 38, 41-42
  variance partitioning 29
Heroin 210
High-activity line selection 14
Human alcohol-drinking behavior 119
Hurnik T-maze studies 65
Hyde aggression studies 67
5-Hydroxyindoleacetic acid 180
Hyperkinesis 168

I

ICR mice 216
Inbreeding
  accidental 7
  avoidance of 272
  coefficient of 14-15
  deliberate 7
Independent culling (*See also* Selection methods) 55
  method 274
Index selection (*See* Selection methods) 56
Individual selection (*See* Selection methods) 46
Initial sensitivity 281
Innate tolerance 181
Intensity of selection equation 39
Intervention experiments 84
Intraclass correlation 47-48
Isoenzymes 236
Isopropanol
  tilting plane performance 136
  tolerance in rats 88, 111

L

Lafayette activity platforms 194
LA strain (*See* Least-Affected rat strain) 114, 195
Lagerspetz mouse studies 64
Least-Affected rat strain
  breeding method 195
  CNS sensitivity 196
  locomotor activity 197
  motor activity 198, 239

  rat studies 114
  weight at birth 198
Least square method 262
Limbic system 219
LISREL computer program 254
Lithium chloride 199
Litter sizes in rats 94
Locomotor activity
  effect of morphine 216, 219
Long-Evans rats 194
Long-sleep mice 193
  adrenocortical response 167
  behavioral response 161, 162
  GABA brain levels 165-166
  morphine response 229
  motor activity 239
  neurotransmitter amines 166
  pharmacological research history 161
  righting response 150
  salsolinol 166
  seizure threshold 167
  voluntary ethanol consumption 165
Low-activity line selection 14
LS mice (*See* Long-Sleep mice) 150, 161

M

MA rats (*See* Most Affected rat strain) 114
Mass selection (*See* Individual selection) 46
Maternal effects 272
Mating speed
  in Drosophila 73
Maudsley Reactive strain 69
Maze-Brightness (*See* Selective breeding programs) 59
Maze-Dullness (*See* Selective Breeding Programs) 59
Mean body weight
  in AA and ANA rats (Table 1) 92
  in AT and ANT rats 156, 161
Meiosis 41
Membrane fluidity 136
Mendelian genetics 3
MEOS (*See* Microsomal ethanol oxidizing system) 162
Metabolic tolerance (*See also* Voluntary ethanol consumption) 126-127
Metabolism 279
Methyl alcohol response curves 163
3-Methyl cholanthrene 162
4-Methylpyrazole 133
Microsomal ethanol oxidizing system
  hepatic 162
Mixed Strain rats
  cross-breeding analysis 93-94
  polymorphic loci 113
Morphine 210
Most-Affected rat strain 114
  breeding method 195
  CNS sensitivity 196
  effect of pentobarbital 200
  lithium chloride sensitivity 199
  motor activity 197, 198, 239
  weight at birth 198
Moving belt test 238
Multiple regression analysis
  correlational theory 251
  step-wise 254
Multiple trait selection 274
Multivariate analysis 249
Mycoplasma pulmonis 95

N

Naloxone effects 211
NP rats (*See* Alcohol nonpreferring) 113
Nachman saccharin studies 65
Neurotransmitter
  in LS and SS mice 165-166
  in P and NP rats 179-180
Newletters 285
Nonadditive effect 41
Norepinephrine
  morphine studies 218
  P and NP rat brain 180

O

Open-field activity
  in AA and ANA rats 122-123, 137
  in LS and SS mice 165

  salsolinol 166
  withdrawal 185-186
Open-field behavior 11
  classic studies 66
  index of emotionality 11
  scoring
    albino mice 14, 23
    automated 12
    defecation 12, 13
    pigmented mice 14, 23
Open-field emotionality 225
Open-field test (*See also* Alcohol sensitivity tests) 96
Operant reinforcement
  AA and ANA rats 181
  P and NP rats 181
  intravenous administration 183
  Sprague-Dawley rats 181
Opiate-alcohol interaction 227-228
Opiate-induced analgesia 205
Opiates
  behavioral responses 212
  drug studies 210
Opposing natural selecion (*See* Response to selection) 50
Oriental population (human) 132
Outbreeding (*See also* Selection method) 112
  purpose of 235

P

P rats (*See* Alcohol preferring) 113
Paraldehyde response 163
Path analysis 31, 252-253
Pathology 282-283
Pentobarbital
  LA and MA rats 197
  LS and SS mice 163, 164
Pentylenetetrazol 244
Phenotypic expression 271
Phenotypic score
  drinking behavior 90
  environmental influence 4
  gene loci 4
Phenotypic standard deviation 39
Physical Dependence 280
Phototaxis 73
Pilocarpine 167
Pleiotropic gene action 271
Polygenic theory 8
Predictor variable 251
Preference testing
  method 174
  operational definition 280
Progeny test (*See also* Selection methods) 47
  method 273
Psychological Dependence 280
Pyrazole
  alcohol withdrawal experiment 165
  ethanol-elimination rate test 235
  inhalation model 245

Q

Quantitative genetic theory
  model
    environmental deviation 23
  genotypic value 23
    phenotypic value 23
Quinine preference 228

R

Racial Studies 172
Random genetic drift
  effect of 271
  non-selected lines 17
  selected lines 21
Realized heritability 44
Rearing behavior studies 65
  scores 261
Reinforcing properties 181
Research Laboratories of the State Alcohol Monopoly of Finland 87
Respiratory rate 240
Response to selection
  accumulated inbreeding 50
  correlated response
    genetic correlation 54
    line differences 81-82
  definition 38
  establishing baseline 44
  experimental results 49
    activity scores 15

  defecation scores 17
 fall-time scores
  in SS mice 158
 genetic asymmetries 51
 LA and MA rats 196
 measuring 44
 opposing natural selection 50
 phenotypic standard deviation 42, 43
 response curve 53
 selection favoring heterozygotes 50
Restraint-time 149, 155
Retrospective selection 275
Righting reflex (*See also* Sleep-time studies) 148, 149
 dose response curves 163
Roderick-Wimer strain 69
Rotarod test (*See also* Alcohol sensitivity tests) 96
 acute intoxication measure 238
 AT and ANT rats 107
RT procedure (*See* Restraint-time) 149
Rundquist

S

Saccharin preference studies 65
 ethanol 198-199
 morphine 222
Salsolinol 166
Scopolamine 167
Seizure threshold 167
Selection (*See also* Selection methods)
 bi-directional 14
 differential equation 38
 experimental design 271
 favoring heterozygotes 50
 limits 52
Selection Methods
 between-family selection 46-47
 independent culling 55
 index selection 56
  restricted 57
 individual selection 46
 outbreeding 112
 progeny tests 47
 sib selection 47
 tandem selection 55
 within-family selection 48, 270, 272
Selection pressure 174
Selective breeding
 agricultural application 6
 alcohol sensitivity 161
 animal models 11
 bidirectional selection 6
 effect in AT and ANT rats 100
 like phenotypic score 4
 maze-brightness 59, 60
 maze-dullness 59, 60
 outbreeding 87, 119-120
 theory 25, 37
Self-administration
 of opiates 220
Serotonin (*See also* Brain serotonin concentration) 126-127
 in P and NP rats 180
Short-sleep mice
 adrenocortical response 167
 breeding 193
 GABA brain levels 165-166
 motor activity 239
 Neurotransmitter amines 166
 pharmacological research history 161
 response to morphine 229
 righting reflex 150
 salsolinol 166
 seizure threshold 167
 sensitivity to ethanol 161
 voluntary ethanol consumption 165
 waking blood alcohol concentration 163
Sib selection (*See* Selection methods) 47, 273-274
SJL mice 216
Sleep-time studies 234
 AA and ANA rat strains 136
 C57BL and BALB mice 147, 148
 LA and MA rats 197

  P and NP rats 181
  righting reflex 149
Sleeping-time test (*See also* Drinking Behavior) 88
  scores 150, 154
Sprague-Dawley rats 89
  acetaldehyde levels 132
  breeding LA and MA rats 194
SS mice (*See* Short-Sleep mice) 150, 161
Step-wise multiple regression 254
Sucrose morphine solution 221
Swimming impairment 199
Swiss-Webster mice
  acute tolerance 243
    dowel test 239
  morphine studies 216
  withdrawal seizures 244

T

Tail-flick test 215
Task group topics 269
Taurine 180
T-Maze performance studies 65
Tandem selection (*See also* Selection methods) 55-56, 274
Tetrahydroisoquinoline 236, 237
Thermal pain response 214
Thompson intelligence studies 61
Tilting-plane test (*See also* Alcohol sensitivity tests) 88, 96
  AT and ANT rats 106
  ataxia 238
Thyroid function studies 69
Tolerance
  AA and ANA rats 199
  breeding method
    acute developing 242-243
    chronic developing 241-242
  difference in rat strains 114-115
  MA and LA rats 199
  operational definition 279
  P rats 186
Tolman breeding studies 59
Trichloroethanol 163
Tryon maze studies 60, 70
Twin studies 172
Tyrosine 180

U

Ugo Basile Rotarod test 98
Univariate sample regression 251
Urinary excretion 122-123, 137

V

Van Abeelen behavior studies 65
Variation of expression 271
VARIMAX program 256
Vasopressin excretion 122-123, 137
Vietnam veterans 211
Voluntary ethanol consumption
  AT and ANT rats 108
    relation to intoxication 136
  AA and ANA rats (Table 2) 92
  effect of age 110
  effect of caloric restriction 185
  effect of sweeteners 165
  effect of tetrahydroisoquinoline 237
  factors regulating 121
    environmental 109
  metabolic tolerance 126-127
  P and NP rats 174
  relation to tolerance 112, 114
  Wistar rats 177

W

Waking blood alcohol concentration 163
Wistar rats 89
  breeding P and NP lines 173
  inbred stains 132
  morphine consumption 220
Withdrawal reaction
  ethanol metabolism 238
  seizure susceptibility 244
Within-Family Selection (*See also* Selection methods) 48
  animal models 272
  divergent 270

8579

Evaluation of the Alcoholic: Implications for Research, Theory, and Treatment

Editors: Roger E. Meyer, M.D., Bernard C. Glueck, M.D., James E. O'Brien, Ph.D., Thomas F. Babor, Ph.D., Jerome H. Jaffe, M.D., and James R. Stabenau, M.D.

Proceedings of a national symposium in October 1979, cosponsored by the University of Connecticut Alcohol Research Center and the National Institute on Alcohol Abuse and Alcoholism. The monograph addresses the general notion of differential assessment as it applies to persons diagnosed as alcoholic. Papers examine the issues and identify promising developments in clinical and research activities.

DHHS Pub. No. (ADM) 81-1033 *Printed 1981*
GPO Stock No. 017-024-00988-1 *Cost: $8.00*

These monographs are available from the Superintendents of Documents, U.S.
vernment Printing Office, Washington, D.C. 20402. Please order by title and GPO
ock Number.

DHHS Publication No. (ADM) 81-1133
Printed 1981

NIH LIBRARY
Amazing Research.
Amazing Help.
http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

WERT
BOOKBINDING
Grantville, Pa
SEPT - OCT 1989
We're Quality Bound

NIH LIBRARY
3 1496 01022 8008